VOLUME III NOVEMBER, 1898 No. 6

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

ON A FORM OF DEGENERATION OF STRIATED MUSCLE MET WITH IN THE UVULA.

BY A. G. HOEN.

(From the Anatomical Laboratory of the Johns Hopkins University.)

PLATES XLII–L.

There is but little literature descriptive of the microscopic morbid anatomy of the uvula. This is true especially of the condition known as relaxed or elongated uvula. Inman* in 1852 described the uvula in this condition as being "very firm and almost fibrous in the interior, covered with ordinary mucous membrane, which was not hypertrophied. On making sections and teasing with needles, the structures were found to consist of groups of large cells, 1/250 inch in diameter, studded with an immense number of granules." These he supposed to be altered cells from the mucous glands, bound together by strong areolar tissue. "Throughout this tissue were interspersed numerous muscle fibres, arranged in bundles, and but few of the fibres had the characteristics of voluntary muscle. Very few were striated; all of them seemed to be composed of an outer membrane and an inner substance, closely resembling fibrin. It was homogeneous, yellow, divided irregularly into fragments by transverse

* *Medical Times and Gazette*, 1852, v, p. 232.

divisions. The majority of the fibres terminated in blunted points as if they were undergoing absorption."

The condition of the uvular tissues found by Inman was, in the main, probably the same as that which I shall describe in this article. It is not unlikely that, owing to the imperfect microscopic technique and methods of staining and examination at the time when Inman wrote, he was unable to study the exact relations of the tissues and to interpret correctly what he saw.

I have recently had the opportunity of studying the uvula microscopically in certain pathological conditions, and of comparing the appearances found with those in normal specimens.

The material serving as a basis for this study consisted of the following: (1) a uvula, relaxed and elongated, removed from a patient in my own practice and fixed for microscopic examination in Zenker's fluid; (2) three uvulæ from the Laryngological and Rhinological Clinic of the Johns Hopkins Hospital Dispensary, removed by Dr. Gamble, which were hardened respectively in saturated mercuric bichloride (aqueous) solution, 5 per cent formalin, and Zenker's fluid; of these three specimens the formalin preparation proved to be practically normal and served as a standard for comparative purposes; (3) a fifth uvula, removed by Dr. Mackenzie for elongation, from a case of chronic pharyngitis in an inveterate smoker, and hardened in graded alcohols; (4) the sixth removed by Dr. Flexner at autopsy from a new-born infant half an hour post mortem and fixed in 5 per cent formalin. Obligations are here expressed to the above-named gentlemen for their courtesy in securing and sending the material as enumerated.

In May, 1896, R. W., æt. 30, presented himself for treatment, fully impressed with the idea that he was suffering with a tubercular affection of the lungs. Mother died of phthisis at 40. Family history otherwise good. Patient is a moderate smoker. Briefly stated, his symptoms were as follows: He suffered from cough which was of an irritating character and incessant, and he had very scanty expectoration of a rather tenacious and glairy mucus. Occasionally this became puriform. No hæmoptysis. He had morning vomiting and he was also sick at his stomach during the day. His temperature in the evening was slightly elevated (99°-99.5° F). The appetite was good, but the irritating cough frequently caused regurgitation or vomiting of his food. His sleep was very much broken by the cough, and he was compelled to use an anodyne cough-mixture. Deglutition was painful, and there was a constant desire to swallow.

Inspection of his throat revealed a moderate erythematous blush involving the whole of the pharyngeal mucous membrane, as well as the pillars and arch of the soft palate. The mucous membrane seemed to be slightly œdematous and presented a moderate degree of capillary injection. The uvula was extremely long and relaxed, and rested, curled up, on the base of the tongue. Examination of the sputum was negative as to tuberculosis; auscultation and percussion demonstrated little of note, a slight harshness on forced inspiration and expiration being the only abnormality perceptible. Circulation sound and renal functions perfect.

As most of the symptoms seemed to point to a reflex origin due to the elongated uvula, abscission of the latter was advised and performed, with prompt relief of the urgent symptoms.

Microscopic Technique.—The removed portion, somewhat over 2 cm. in length, was immediately placed in Zenker's fluid, where it remained for 36 hours. It was then transferred to 80 per cent alcohol, and finally run through absolute alcohol, thin and thick celloidin, in which it was imbedded. Longitudinal sections were made. An oversight occurred in neglecting to treat the sections first stained and mounted with iodine solution, in consequence of which these show precipitates of the mercurial salts.

Various staining methods were employed, Delafield's hæmatoxylin solution, and one* which I have employed for some time in this laboratory, giving on the whole the most satisfactory specimens for general purposes. The sections stained in Delafield's hæmatoxylin were in some instances differentiated by a short immersion (from 1 to 2 minutes) in an aqueous solution of oxalic acid (1 per cent); in others, in a saturated solution of picric acid in water for the same length of time. Sections stained in the glycerine-Heidenhain-hæmatoxylin mixture require no differentiation, as it does not overstain, and is quite elective in its action, requiring, however, from 12 to 24 hours before staining is complete. For counterstaining a saturated alcoholic (95%) solution of ammon. picrate, to a watchglassful of which a few drops of a 2 per cent aqueous mixture of liq. ammoniae were added, was used, the sections being transferred from the acid differentiating bath directly to this. Eosin-creosote (from 2 to 4 per cent sol.) was also used as a counterstain and as the final clearing agent for some of the sections, but the ammonium picrate treatment gave by far the most satisfactory and instructive specimens.

* Glycerine, 50 cc.; Heidenhain's hæmatoxylin sol., 20 cc.; alcohol, 95 per cent, 100 cc.; water, 50 cc.; ammon. alum, 2-4 grammes.

The sections were transferred directly from the ammon.-picrate-alcohol solution to the slide, where they were dried with filter paper, and immediately flushed with xylol-anilin for a second or two, then with pure xylol, and finally mounted in balsam. Terebinthinated creosote, cedar and clove oils were also employed as clearing agents, and after special staining methods. Weigert's fibrin stain, van Gieson's picric acid-fuchsin, Ehrlich's triple stain and thionin in 1 per cent solution (aqueous) were also employed, the two latter with the special end in view of determining, if possible, the nature of the granula found in many of the cells which were abundantly scattered and grouped throughout the tissues under consideration.

While the following description is based mainly upon the appearances found in the uvula obtained from the patient whose clinical history has already been cited, the five others which were obtained for comparative and corroborative examinations have also been carefully studied and will occasionally be referred to in what follows.

It seems desirable, before entering into the details of the degenerative process under consideration, to give a brief description of the tissues of the uvula in general.

The epithelial covering of all of the uvulæ (excepting the one from an infant) seemed to be slightly thickened; the anterior (oral) surfaces were corrugated, and in the one designated as "smokers' uvula" the corrugations were most marked. Posteriorly (pharyngeally) their surfaces were comparatively smooth, and the layer of epithelial cells was much thinner than that of the anterior surfaces (Plates XLII-XLIII). In both regions the nuclei of the epithelial cells presented in large number the various phases of typical mitotic activity. No ciliated epithelium was found. Careful examinations of many sections of the different uvulæ failed to disclose any evidences of either recent acute or chronic inflammatory changes in the epithelial covering (unless the number of mitotic nuclei or the seemingly slight thickening, already alluded to, should be looked upon as pathological), and in every instance the epithelial covering was intact.

General leucocytic invasion (phagocytosis) or accumulation of cells of this type in localized foci in the tissues of these uvulæ was never observed, though in all (save in the one from an infant) was found a form of granular cell which morphologically appeared to be identical with the Mastzelle of Ehrlich (Plate XLIX, Fig. 15). The granular cells

failed, however, to give uniformly the decisive color reaction with the basic anilin dyes, which is a distinguishing characteristic of such cells. Once, in the formalin uvula, the granules of these cells reacted specifically, after staining with Nissl's methylene-blue sol., becoming purplish red, whilst the nucleus assumed a rather pale blue color. With thionin (which is recognized not only as an elective stain for mucin, but also as one for the granules alluded to above) there was no metachromatic reaction, granules and nuclei being stained alike and both very intensely. These cells were found in numbers in the lymph spaces of the connective tissue, the sheaths of the blood-vessels, the septa of the gland-lobules, the adipose tissue, and the perimysial tissue. As the result of pressure from surrounding parts, they presented varied shapes, the round and ovoid predominating, the nucleus being sometimes in the centre and sometimes at one end of the cell. As found between the muscle fibres, they were often elongated, the granules being disposed in a narrow band, gradually tapering to a point at either end.

Not infrequently there were found in the lymph channels of the delicate tunica propria lymphoid cells, which showed karyokinetic changes in several different phases, the most common being the monaster (mother star) and the well advanced dyaster (daughter star). These interesting histological phenomena were noticed only in the uvulæ fixed in mercuric bichloride solution, the most carefully instituted search in other uvulæ failing to reveal them. The sections showing these figures were those which had been stained in a 1 per cent solution of thionin in distilled water for a few minutes, decolorized in alcohol of various strengths and finally mounted in balsam. They are probably not abnormal appearances.

The lymph capillaries and spaces in both the tunica propria and the submucosa presented evidences of crowding with lymph cells, which at some points was more pronounced than at others. The presence of these lymphoid cells was particularly noticeable in the perivascular lymph sheaths as well as in the delicate connective tissue of the tunica propria which enters between the papillæ of the mucous membrane, and in a few instances actual lymph nodes were present in these regions. In the deeper layers of the submucosa the lymphoid cells were not so numerous.

Many of the granular cells already mentioned were found scattered throughout this latter tissue. Within many of the lobules of the mucous glands, with which the uvula is well supplied, lymphatic follicles were frequently observed in close apposition to the glands. These lymph nodes exhibited the characteristic denser cortical portion and the lighter central areas, corresponding to the germinal centres. The lymph chan-

nels going to and passing from such nodes could be easily traced in the intertubular connective tissue septa by the lymph cells which they contained. Such appearances are doubtless entirely normal.

All of the uvulæ were found to be richly supplied with blood-vessels, of which the larger were found in greater number at the periphery in the submucoid connective tissue, where they formed a network completely investing the uvula. The main and largest blood-vessels, which were few in number, pursued a central course, longitudinally, through the uvula, giving off branches at intervals, which again subdivided to form the capillary system of the various tissues situated in the interior of the organ. The tunica propria obtained its capillary blood supply from the peripheral blood-vessels already alluded to.

The blood-vessels, both large and small, as well as the capillaries, presented no noticeable abnormalities in any of the uvulæ. Ruptured blood-vessels, hæmorrhages or extravasations were never noticed. In the blood-vessels of the uvula fixed in formalin curious egg-shaped formations were noticed, of a clear homogeneous structure, which were arranged peripherally and seemed to be attached to the endothelial cells lining the vessel walls (Plate XLVI, Fig. 1). The blood corpuscles in these vessels, as a rule, were crowded to the centre, filling up the rest of the lumen of the blood-vessel. This appearance was noticed not only in this uvula, but also in one fixed in alcohol, and undoubtedly represents an artefact, produced by the fixing agents employed.

The uvula is well supplied with glandular tissue. The mucous glands were found to occupy the upper third or proximal portion of most of the uvulæ examined (Plate XLII). The interacinous connective tissue contained single fibres of striated muscle, and in some instances bundles of four or five such fibres could be seen running between the lobules of the glands. This arrangement of the striated muscle fibres was particularly apparent in the uvula obtained from an infant at autopsy. Fat cells, in groups of from five to fifty or more, were also noticed interspersed in the connective tissue septa between the lobules of the glands, along with the gland ducts, many of which were filled with secretion.

In one of the uvulæ, which will be spoken of as "smokers' uvula," a most remarkable hyperplasia of the mucous glands was apparent, which could be seen to extend as a continuous chain from the point of abscission down to the tip or distal end of this uvula (Plate XLIII). In every instance, as is apparent in Plates XLII and XLIII, these glands occupied positions relatively nearer the posterior or pharyngeal surface than the anterior or oral. Most of the cells lining the tubuli of these glands exhibited a finely granular condition of their protoplasm. The uvula

hardened in mercuric bichloride solution showed this granular condition in a most marked degree. In one of the sections made from a formalin preparation and stained in dahlia solution for twenty-four hours, well decolorized in alcohol and subsequently stained with methylene-blue, it was noticed that here and there isolated tubuli retained the dahlia stain with great pertinacity, the remaining tubuli having given up the latter stain and taken the blue quite electively.

Snarls and wisps of nerve tissue were found here and there cut in various directions, transversly, obliquely and longitudinally, and appeared to be normal in every respect. The myelin or medullary sheath of those found in the uvula fixed in mercuric bichloric solution appeared distinctly and coarsely granular, for which condition the hardening fluid used was undoubtedly responsible. Ganglionic nerve cells were not recognized in any of the uvulæ examined.

The general connective tissue of the uvula appeared to be more or less increased in all excepting the infant's uvula (autopsy), and was least marked in the one hardened in formalin. White fibrous connective tissue seemed to predominate throughout, the finer threads of the elastic variety being less common. These elastic fibres were easily demonstrable by a prolonged (over night) immersion in a .1 per cent solution of methyl violet, which stained them with precision and great intensity.

In the uvula hardened in alcohol (smokers' uvula) the hyperplasia of the connective tissue was much more advanced than in any of the others, and in many places its resemblance to scar tissue was very pronounced, it being but sparsely supplied with blood-vessels and almost entirely devoid of nuclei. van Gieson's picric-acid-fuchsin mixture stained it intensely.

CHANGES IN THE STRIATED MUSCULAR TISSUE.

At the point of abscission most of the muscular fibres showed very distinct, even exaggerated, transverse and longitudinal striations. The cross striations seemed coarser and the interfibrillar spaces wider than in a normal fibre, while the whole fibre presented an œdematous and swollen appearance (Plate XLVI, Fig. 2). Fibres of diminutive calibre at this point of the uvula might be looked upon as longitudinal peripheral segments of fibres which were only partially in the plane of the section. Here and there, but only at this part of the uvula and at their cut ends, fibres may be found which present somewhat the appearance of Zenker's waxy degeneration. As the resem-

blance to this form of degeneration was observed only here, it is probable that the surgical trauma inflicted in the removal of the uvula was responsible for its appearance, as Erb* and Weber† have demonstrated this experimentally with wounded muscle of living animals.

Plate XLVI, Fig. 2, shows two œdematous and swollen fibres with exaggeration of their cross and longitudinal striæ, and near the end of one of them a nuclear invasion which will be noticed further on under the heading "Nuclei of the Muscular Tissue." Following these fibres towards the distal or free end of the uvula, others were found in which peculiar absorptive and atrophic phenomena begin to manifest themselves, accompanied by what will be named (for want of a better designation) a peculiar bleb-like or vesicle-like homogeneous "deposit" at their peripheries (Plates XLIV and XLV), each vesicle, as a rule, containing a nucleus. A description of these contained nuclei will be given later.

In the beginning this deposited material may show only as a narrow, bright margin or line of a homogeneous nature along the periphery of the affected fibre, giving to the latter a double contour; or it may appear at irregular intervals, the sarcolemma being apparently raised here and there into small blebs or blisters containing the material referred to above (Plates XLVII, Fig. 7, and XLIX, Fig. 17).

At this stage of the alteration transverse striation is still distinctly visible, though not in so marked a degree as in those fibres which have been previously described. Longitudinal striation has been replaced by wavy and undulating lines corresponding to the individual fibrillæ which have now become twisted upon themselves, presenting an appearance not unlike the strands of a rope. This appearance is most noticeable at the free ends of those fibres which have advanced to this degree of the degeneration (Plate XLV). Some stress may be permitted in directing attention to this phenomenon of fibrillar twisting, in that it becomes, as a rule, apparent only after the marginal change begins to manifest itself.

* Virchow's *Archiv*, xliii (1868), 108.
† Ibid., xxxix (1867), 216.

In some of the fibres, immediately at their periphery and between these and the vesicle-like structures, individual fibrillæ appear, which are breaking up into fine granules, yellowish in color, and not affected by stains, granules which under high amplification (× 1100) present the appearance of pigment (Plate XLVII, Fig. 6).

More or less opacity and a brownish yellow color are noticeable, in unstained sections, in all of the fibres which are well advanced in the degenerative process. Transverse cleavage into disks or blocks was noticed in only very few fibres, and then immediately in the neighborhood of the line of abscission. The same may be said of tortuous or kinked fibres, a few of which were found only at the point of surgical interference. van Gieson's picric-acid-fuchsin stained all fibres deeply and diffusely, as did eosin.

The final stage of the peculiar degenerative process is reached when a veritable cylindrical plug or mass composed of large blebs, containing small and large misshapen nuclei intermingled with pigmentary detritus, is formed, through the middle of which runs longitudinally a mere shadow, suggestive of the former site of a muscular fibre (Plates XLVII, Fig. 8, and XLVIII, Figs. 9 and 11). Occasionally such a mass may be found in which one, two, or more fibrillæ may still be distinctly visible with well preserved transverse striæ. Fibres were also observed which presented an unequal degree of degeneration, one end being only partially affected, whilst the other revealed the fibrillæ gradually being absorbed in a tapering fashion until they assumed a wedge shape, with a single projecting fibrilla, the whole being surrounded by cell-like vesicles containing nuclei (Plate XLIX, Fig. 13).

Examination of transverse sections of fibres shows in many a want of uniformity of distribution of the " deposited material " that is, one-half only of a fibre may be involved, the other half remaining apparently normal (Plate XLVII, Fig. 5). Again one may see such a semi-degenerated fibre showing a few intact fibrillæ in the centre of the degenerated area.

In the transverse sections made from the uvula hardened in mercuric bichloride solution (Plate XLVIII, Fig. 12), a peculiar condi-

tion of the contents of the blebs was brought about by the action of this fixing agent; instead of the clear and homogeneous character of the material which was contained in the blebs of other uvulæ treated with formalin, Zenker's fluid, and alcohol, a granular precipitate, more or less fine, was noticeable, which in some of the cross-cuts of fibres was arranged radially, running from the remaining portion of the fibre in the centre of the bleb to what should represent the sarcolemma at the periphery. Subjected to a prolonged staining (24 hours) with Ehrlich's triple stain, the granular deposit took the fuchsin of the above mixture, the fibres, or what remained of them, taking both this and the orange with great intensity.

Although our material was hardly sufficient for the purpose, we have utilized it in order to fix the diameters of the muscular fibres with a view of determining whether hypertrophy existed. There is no literature extant indicating research on this particular point as regards the uvula. In an exhaustive and elaborate article by R. Mayeda,* an effort has been made to establish a scale of measurement for the different muscles of vertebrates. It is sufficient to state here that the conclusions arrived at are such that not only is it necessary to select muscles for comparative measurements which are anatomically identical and from the same species, but the conditions of nutrition and general health must also be taken into account in making such calculations.

Of the six uvulæ examined, from which the infantile uvula, on account of the primitive development of its muscle fibres, must be excluded, only one (the formalin specimen furnished by Dr. Gamble) could be used for the purpose of supplying a standard for making comparative measurements. This uvula may be considered as being practically normal with reference to all of its tissues, and its muscle fibres presented an average diameter of 0.019 to 0.023 mm. (Plate XLVI, Fig. 1). The uvula hardened in Zenker's fluid, with subsequent alcohol treatment, presented fibres which measured from 0.035 to 0.040 mm. (Plate XLVI, Fig. 2). The one hardened in merc. bichlor. sol. (Dr. Gamble) presented fibres which measured up to 0.050 mm. The peculiar muscular degeneration in this uvula was

* *Zeitschr. f. Biol.*, n. F. ix (1890), 119.

very marked, every fibre which appeared in the sections (all of which were transverse) showing it (Plate XLVIII, Fig. 12). The measurement of one of the largest of these fibres (already stated as 0.050 mm.) was made without including the peculiar marginal "deposit" already described. If the "deposit," which presumably is limited by the sarcolemma on the one side, and what represents the true muscle fibre be included, the measurement is brought up to 0.075 mm.

Nuclei of the Muscular Tissue.—Proliferation and polymorphism of the nuclei, characteristic of many forms of degeneration and of other lesions of muscle, are most prominent phenomena of the peculiar metamorphosis here under consideration. The shape, size and position of the nuclei, however, are so varied that a detailed description is necessary in order to give a comprehensive idea of them.

Fibres which are incipiently affected show proliferating nuclei of nearly normal shape, but somewhat enlarged; and as long as the nuclei proliferate unresisted and can preserve certain measured distances between themselves, they retain their normal shapes (Plate XLVI, Fig. 3). At such points, however, where they become crowded they assume flattened or irregular shapes and may leave their line of proliferation, either dipping down into and among the fibrillæ or becoming heaped up at one end of the fibre into an irregular mass (Plate XLVI, Fig. 2).

Noticeable also were nuclei flattened at their ends and hollowed out at their central portions, on either side, giving them an appearance which much resembled empty cotton spools. Plate XLVI, Fig. 4, presents this condition better than any description. Whether this long string of proliferated nuclei as depicted in this figure represents the dividing line between two fibres which are in juxtaposition, or whether they occupy a position in the centre of a single fibre, could not be determined by microscopic examination of the specimen, as the fibre was immediately lost in glandular tissue on the one hand and in areolar on the other, without giving satisfactory evidence as to this point.

Long, large nuclei, pointed at their ends with their bodies crenated or serrated, the serrations corresponding accurately to the transverse

striæ of the fibre (if one imagines the striæ as projected through the narrow zone of homogeneous protoplasm which surrounds them), were frequently observed. These nuclei corresponded closely to the description given by Martius and Hansemann* of those found in the voluntary muscles in a case of myotonia congenita intermittens. The nuclei in our cases, however, were not so numerous, nor were they found in such long strings as in the case described by these writers. They were for the most part discretely scattered over fibres which still retained many of the attributes of normal fibres, such for instance as those which were situated high up in the proximal portion of the uvula.

The structure of the nuclei, with the exception of their crenated surfaces, was fairly well preserved, chromatin granules and the nucleoli being tinged almost perfectly by the proper stains.

Nuclei were frequently found with their long axes placed transversely to that of the fibre and with a tendency to become irregularly disposed.

The majority of the nuclei show, completely surrounding them, a more or less extended halo of a clear and homogeneous material, not affected by stains of any kind or combination (mercuric bichloride specimen excepted), and which seem to be limited by a very delicate and structureless membrane (Plate XLIX, Fig. 17). Fibres which begin to show this erratic disposition and shape of their nuclei are on the verge of manifesting the "bleb-like deposit" referred to or are already doing so. Along the periphery of such fibre nuclei may be seen around which the peculiar bullæ of "deposit" can be discerned in greater or less quantity. Occasionally an empty bleb may be seen, which may be accounted for by the plane of the section having been either above or below that of the nucleus (Plate XLIV).

As the atrophic and absorptive process in the fibres progresses, the blebs or vesicles increase in size, the nuclei retaining their position relatively to the centres of the blebs (Plates XLIV and XLV).

At a still later stage may be seen, in many of the blebs, in addition to the nucleus, a granular material, yellow in color, resembling pigment, which refused absolutely to stain with any dye, save the pig-

* Virchow's *Archiv*, cxvii (1889), 587.

ment found in the uvula fixed in merc. bichloride, and then only after a prolonged action of the staining fluid (Ehrlich-Biondi stain, 24 hours) (Plate XLV; Plate XLVIII, Fig. 9). With the gradual disappearance of the muscular fibre this pigmentary material augments in amount, depositing itself on and closely around the nucleus, which latter shows evidences of decreasing size and of irregularity of contour, with a tendency to fragmentation (Plates XLVII, Fig. 8; XLVIII, Fig. 9). At this stage one may observe here and there a vesicle or bleb in which its nuclear constituent has been entirely supplanted by pigmentary material, and in the final stage, when the fibre has been completely destroyed, its former site being occupied only by vesicles or blebs, the pigmentary deposit alone may mark the site of the original nucleus (Plate XLV). As a rule the pigment granules arrange themselves in two or three small clumps, irregularly in the centre of the bleb, though at other times the whole of the latter may appear to be peppered with this finely granular material. Now and again, however, the granules show a decided tendency to symmetry in arrangement, appearing as rosettes in some of the blebs and stellar-shaped in others. Their absolute indifference to stains serves to distinguish them from nuclear matter, the smallest portion of the latter usually staining deeply.

The nuclei now under consideration in connection with the advent of the pigmentary deposit are characterized by their irregular outlines, having for the most part lost their elongated oval form as well as all detail of structure, the great majority staining solidly and intensely. They appear sometimes as triangular bodies with sharp points; at other times as more or less rounded forms indented or honeycombed at their margins. Again, nuclei were observed shaped like blocks with sharp indentures at their peripheries, giving them a zig-zag or ragged appearance. Knobbed forms also came under observation. Here and there the nuclei failed to stain well, appearing of a pale azure with hæmatoxylin, showing in their interior one or two chromatic granules of a slightly darker shade.

Crescentic nuclei were frequently encountered enclosed in delicate vesicles of nearly spherical shape, the former appearing with their

convexities closely applied to one segment of the vesicle and the whole body somewhat resembling in shape the crescent-shaped organisms found in the æstivo-autumnal type of malarial fever (Plate XLIX, Fig. 17). Nuclei of this shape were observed singly, in pairs and sometimes in greater numbers (five or six), arranged in a row or string, always at the periphery of a fibre, into which they seem to have sunken, and with their vesicular side turned outwards. Their occurrence is synchronous with the earlier stages of the degeneration. Optically, the delicate bleb or vesicle which encloses these nuclei seems to be of the same nature as that which constitutes blebs found elsewhere in the periphery of muscular fibres and already described.

Now and then a vesicle may be found which contains two or more irregularly shaped nuclei closely packed together; examination with high amplifications (800-1100) favors the probability that this appearance is due to the coalescence of two or more blebs. Plate XLIX, Fig. 14, illustrates this appearance, the centre of the field being occupied by a large apparently single bleb containing five deeply stained nuclei.

Evidences of regeneration of the muscle fibres of the uvulae were never observed. Such interesting histological phenomena, however, were kept in mind during the careful examination of some hundreds of slides, and it seems, in the absence of such regenerative efforts, that the muscular degeneration which has been described may be regarded as the cause of the prolapse and elongation of the uvula. The great hyperplasia of the mucous glands found in the specimen designated "smokers' uvula" in the text and shown on Plate XLIII, is probably analogous to similar glandular hyperplasiæ which occur in the pharyngeal mucous membrane in certain chronic inflammatory states.

The presence of muscular fibres within the glandular lobules suggests that they may exert an expulsive influence over the secretory contents of these glands.

I desire to direct special attention to the peculiar character of the changes in the striped muscle of the uvulæ described in this article and clearly demonstrated in the photographs. I have not been able

to learn that identical changes have hitherto been described. As appears from the description which has been given, I regard these changes, although they are associated with marked nuclear proliferation, as essentially degenerative in character, their final stage being a nearly or quite total disappearance of the contractile substance of the affected muscular fibre. The frequency, the anatomical and physiological significance, and the satisfactory interpretation of these muscular alterations can be fully determined only by further investigation of a larger and more varied material.

The technique and apparatus employed in the production of the photographs with which this paper is illustrated have been fully described in the *Bulletin of the Johns Hopkins Hospital*, Nos. 62-63, May and June, 1896.

In conclusion, obligations are here acknowledged to Drs. W. H. Welch and L. F. Barker for their interest and for valuable suggestions during the course of this investigation.

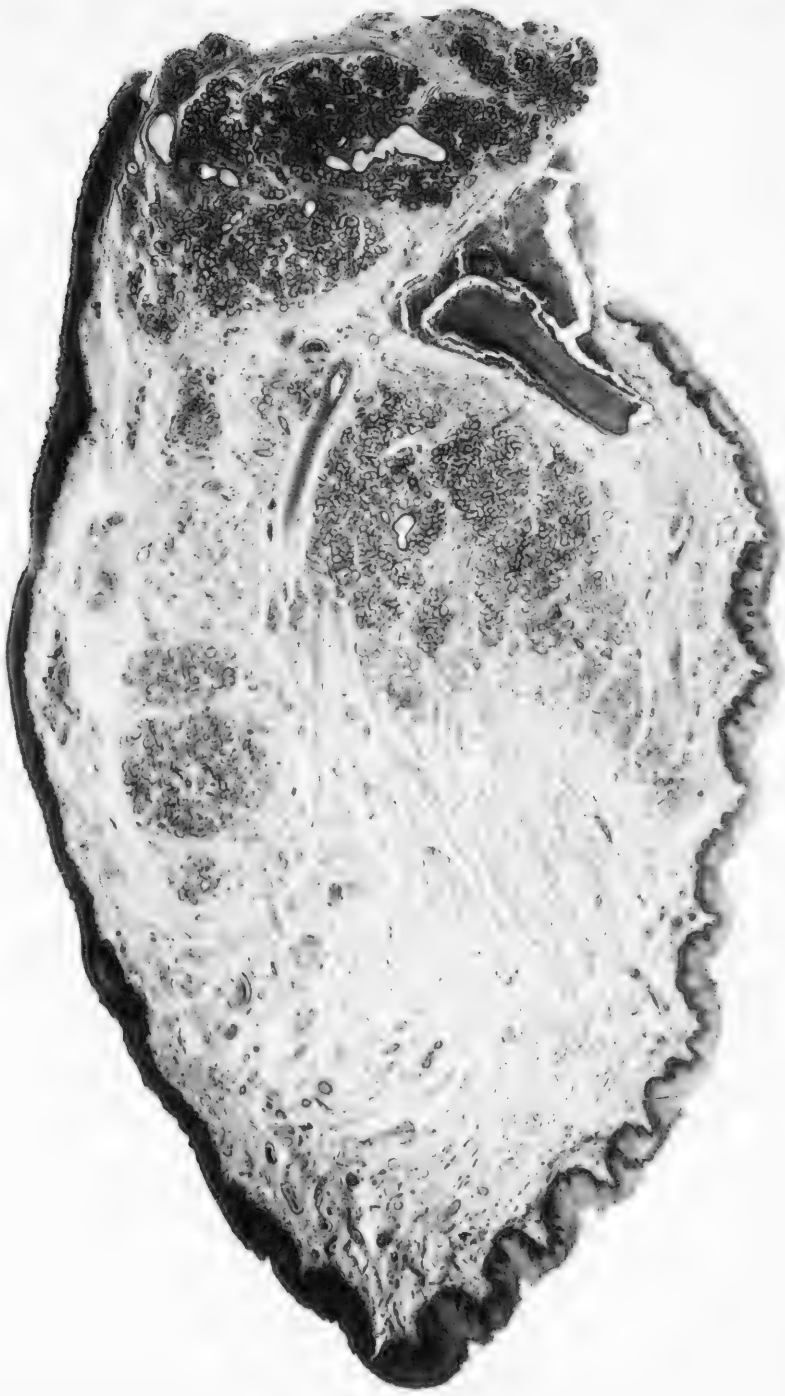
DESCRIPTION OF PLATES XLII-L.

PLATE XLII.

This photograph shows a longitudinal section (sagittal) at about the middle of the entire removed portion of the uvula. The thicker and corrugated mucous membrane to the right represents the anterior surface. The mucous glands occupy, for the most part, the upper or proximal portion of the uvula, although a few scattered patches may be seen lower down. The uvula is well supplied with blood-vessels, the greater number being found near the surface in the areolar tissue just beneath the mucous membrane.

The muscle fibres may be seen as narrow bands in the middle portion of the section, radiating in various directions, the anterior surface receiving a greater number than the posterior. The connective tissue, which was abundant, consisted of dense white fibres, arranged in fasciculi, its greater development being in the region of blood-vessels, around and between the lobules of the mucous glands.

Zenker's fluid preparation. Amplification = Zeiss 70 mm. = × 17½.



THE HELIOTYPE PRINTING CO. BOSTON.

THE HELIOTYPE PRINTING CO., BOSTON.

Plate XLIII.

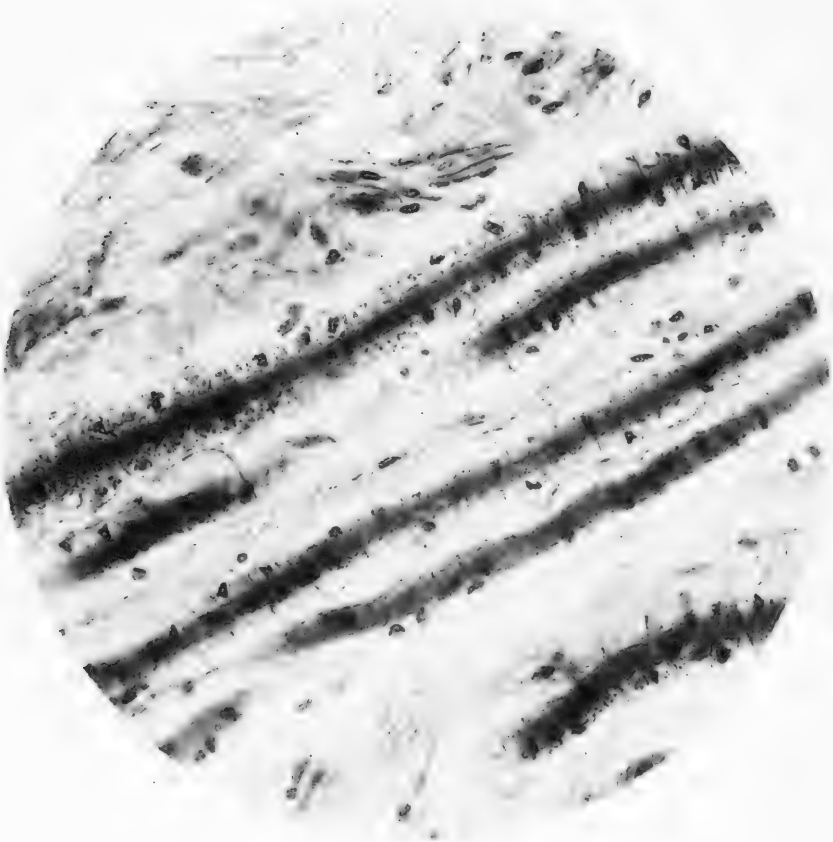
"Smokers' uvula." Sagittal section through about the middle. There is great hyperplasia of the mucous glands, which may be seen as a continuous chain extending from the line of ablation to the tip of the uvula. Muscle fibres have entirely disappeared from the lower two-thirds of this uvula, being replaced by dense bands of connective tissue which ramify around and between the gland lobules, anastomosing at many points. The tissue is very sparsely nucleated and in some places approaches scar tissue in appearance. At the point of abscission a few muscle fibres were found, which, under a high power, did not appear to deviate much from the normal, a slight nuclear proliferation being the only departure. The irregular outline of this uvula is probably due in part to contractions caused by the connective tissue and in part to the action of the alcohol used in hardening. The surface to the left is the posterior or pharyngeal, that to the right the anterior or oral aspect of the uvula. The gland ducts are dilated, and the anterior epithelial covering is somewhat hypertrophied.

Amplification = Zeiss 70 mm. = × 17½.

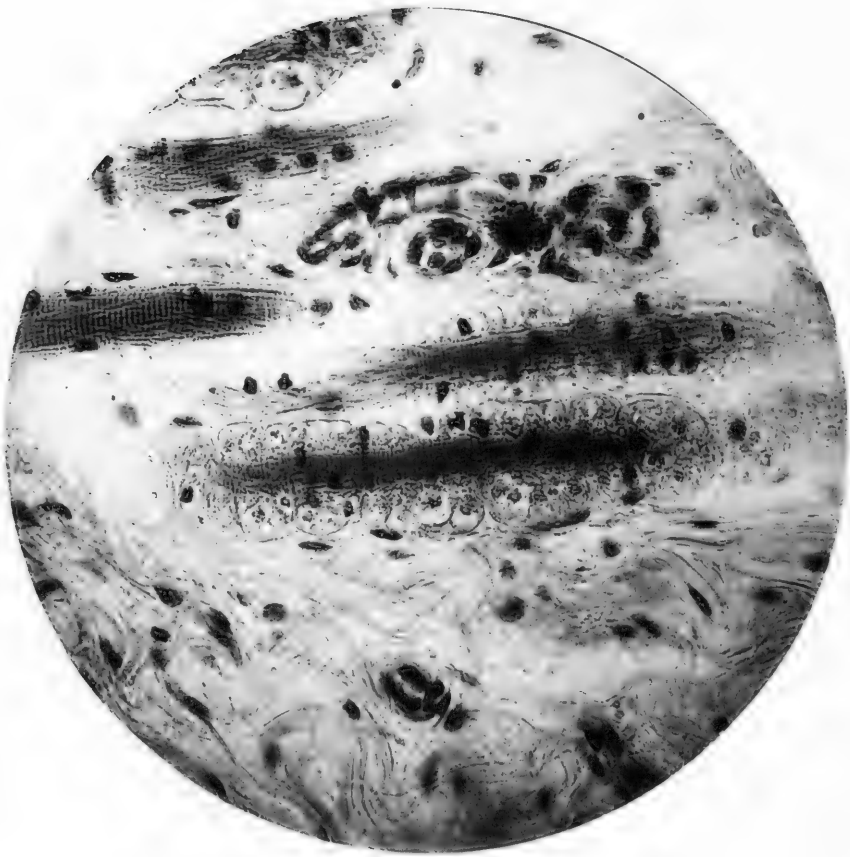
PLATE XLIV.

This photograph shows five muscular fibres, parallel to each other, which exhibit the vesicular or bleb-like process typically, as well as the granular disintegration of some of the individual fibrillæ. Various phases of nuclear change are also well exhibited together with the distribution of granular pigment. For a more detailed description see text.

Amplification = Zeiss 4 mm. Apochromatic, N. A. 95. Compensat. proj. ocular No. 4. Camera length = 550 mm. = × 550.



THE HELIOTYPE PRINTING CO. BOSTON.



THE HELIOTYPE PRINTING CO. BOSTON

PLATE XLV.

Four muscle fibres show advanced degeneration. The twisted condition of the fibrillæ is prominent, the transverse striæ remaining clearly defined. Many blebs surrounding the fibre in the centre of the field contain pigmentary deposit in lieu of nuclei.

In the lower segment of the field wavy connective tissue is apparent, its cells sending out long ramifying processes. It stained deeply with van Gieson's picric acid and fuchsin solution.

Amplification = Gundlach achromat. 1/5. Project. ocular No. 4. Camera length 550 mm.

PLATE XLVI.

Fig. 1. This figure shows in the lower half of the field four or five muscle fibres, which are almost normal save that the individual fibrillæ show a slight tendency to disarrangement. It is difficult to clearly differentiate, excepting at one or two points, the sarcoplasmic, endomysial and endothelial nuclei. The clear space in this specimen is a fat cell; just above this there is an arteriole in transverse section containing clear oval bodies, probably artefacts, which are closely attached to the inner wall of the vessel; they were observed in every blood-vessel of this uvula and in the one fixed in alcohol (smokers' uvula), but in none of the others. This uvula was fixed in 5 per cent formalin with subsequent hardening in alcohol. The muscle fibres measured from 0.019 to 0.22 mm.

Amplification = Gundlach achrom. 1/5. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 2 shows two fibres swollen and œdematous; in a portion of the one at the left part of the field an aggregation of deformed nuclei may be discerned packed closely together. In the same fibre and opposite the nuclei, one or two wavy fibrillæ may be seen slightly lifted away from the rest, a tendency to a general disarrangement of the latter being manifest in both fibres shown. The transverse striæ are sharply defined. These fibres measured respectively 0.035 and 0.039 mm. in diameter. Stained with gentian violet in anilin water—xylol-anilin, xylol, balsam.

Amplification = Gundlach achromat. 1/5. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 3. The muscle fibre here shown approaches the normal, a nuclear proliferation at either end and a slight increase in calibre marking the departure. This is the inaugural stage of the degeneration. With a little care, not only the ordinary transverse striæ, but also the intermediary, secondary, or "Dobie's lines" (in the light discs) may be distinctly made out. The large dark mass seen on the fibre is a precipitate from the fixing fluid used (Zenker's). The dark masses to the right are portions of the mucous glands in cross sections.

Amplification = Gundlach achromat. 1/5. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 4 shows a row of proliferating nuclei; in shape they resemble empty cotton spools. This form is especially apparent in five or six of the nuclei situated in the middle of the string where they are in perfect focus. The clear space to the left of the fibre is a vein cut rather obliquely. The rest of the field is occupied by mucous glands.

Amplification = Gundlach achromat. 1/5. Zeiss project. ocular No. 4. Camera length 550 mm.

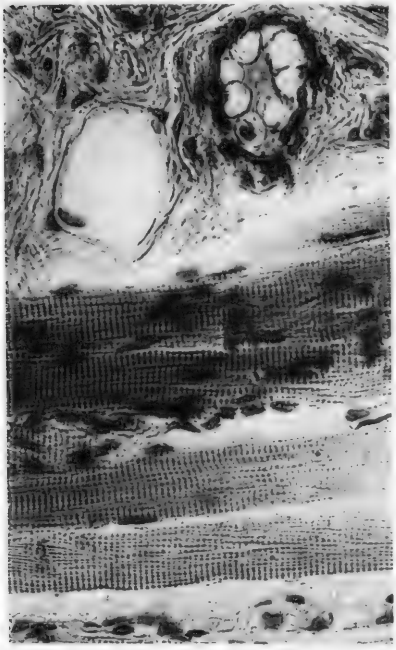


FIG. 1.

FIG. 2.

FIG. 3.

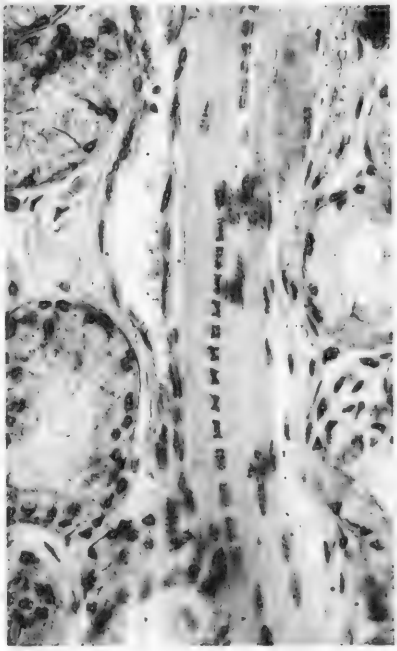


FIG. 4

THE HEINTZEL PRINTING CO., BOSTON.

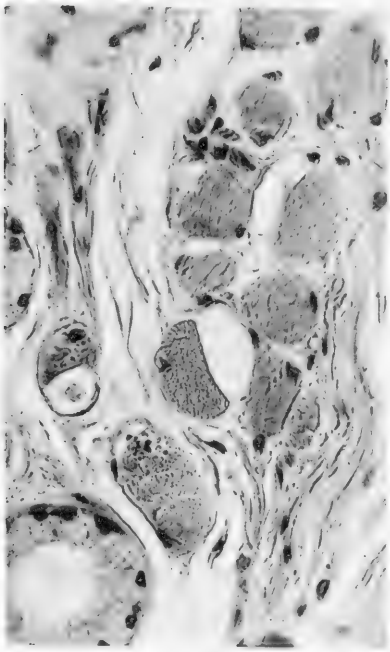


FIG. 5.

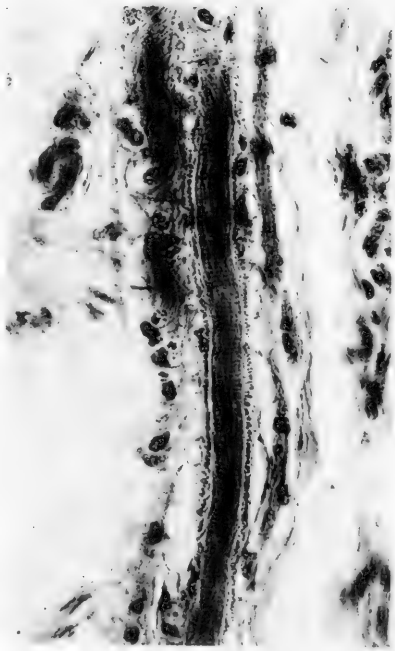


FIG. 6.

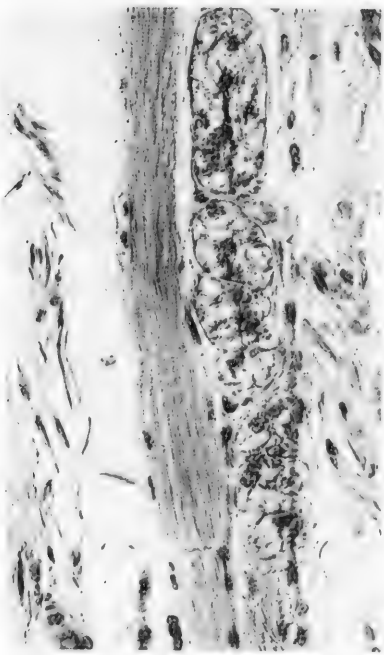


FIG. 7.

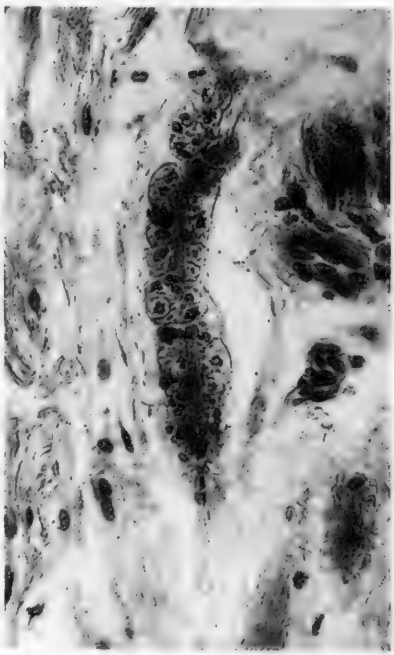


FIG. 8.

THE HELIOTYPE PRINTING CO. BOSTON

PLATE XLVII.

Fig. 5 shows a number of muscle fibres in cross section. The isolated fibre, to the left of the centre, shows the semi-degenerated condition mentioned in the text. The clear homogeneous material, with a few remaining fibrillæ in its centre, occupies one-half of the fibre. A nucleus is seen within the fibrillæ of the remaining half. Diagonally below this a large fibre appears, the fibrillæ of the upper portion of which are disarranged and coarse. Part of a nucleus is visible within them. "Cohnheim's fields" come clearly to view in the rest of the fibre. At the centre of the field a half-moon-shaped fibre may be noticed, closely adhering to which is a perfectly clear and homogeneous vesicular body of similar shape and size to the fibre with which it is associated. Like bodies, though smaller, may be seen in connection with other fibres in the field. Between two fibres, near the upper margin of the figure, a nuclear proliferation in the perimysium internum has taken place.

Amplification = Gundlach achromat. ⅕. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 6 shows a fibre from either side of which a fibrilla has been separated which is breaking up into fine granules, seemingly in accordance with the lines of the transverse striæ. Immediately to the left of this fibre is a fibre the middle portion of which has apparently been totally destroyed, containing a few fragmentary nuclei (karyorhexis) which mark its former site.

Amplification = Gundlach achrom. ⅕. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 7 shows two muscle fibres which extend entirely through the centre of the field. The one to the right has been completely metamorphosed into a mass of blebs; in the extreme upper part of this mass and in its middle a short, thick and dark line may be seen, which represents the last few remaining fibrillæ. The fibre to the left shows constrictions at intervals throughout its length (which may also be noticed on the other one), but it still retains many of its normal attributes. The constrictions are due apparently to delicate connective tissue processes which encompass the fibres at the points mentioned.

Amplification = Gundlach achrom. ⅕. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 8. In the centre of the field is a cylindrical mass composed wholly of blebs; some of these contain nuclei of varying shape and size, several of which are interesting in that they present, if carefully examined, appearances somewhat like the "monaster" stage of dividing nuclei. Some blebs contain pigmentary deposit only, while in others there is also nuclear material. The darkened middle portion of the mass, seen indistinctly through the blebs and extending the entire length, marks what is left of the degenerated muscle fibre.

Amplification = Gundlach achrom. ⅕. Zeiss project. ocular No. 4. Camera length 550 mm.

PLATE XLVIII.

Fig. 9. Near the centre of the field is a mass of blebs containing nuclei and pigmentary material; the dark central shadow in this mass represents what remains of the muscle fibre. At the upper end of the mass are several blebs which contain pigmentary material only, the nuclei having disappeared. Above is a blood-vessel in transverse section. To the right are portions of fibres in various stages of degeneration.

Amplification = Gundlach achrom. 1/5. Zeiss project. ocular No. 4. Camera length 500 mm.

Fig. 10 shows a section of the uvula described as "smokers' uvula" in the text, and is introduced to show the very dense nature of the connective tissue, which in connection with glandular hyperplasia represented very nearly all of the tissues of which this uvula was composed. This tissue contained few cells of any kind (fat cells excepted), and its vascular supply was also small. The fat cells are represented by the clear spaces.

Amplification = Gundlach achrom. 1/5. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 11. In the centre of the field a fibre is shown which has been almost completely metamorphosed into blebs; a narrow dark band running through the middle of the mass indicates the remains of the muscle fibre. The clear space partially shown in this field is a portion of a fat cell.

Amplification = Gundlach achrom. 1/5. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 12 is a transverse section of the muscle fibres from the specimen hardened in mercuric chloride solution. The staining (Ehrlich-Biondi, 24 hours) was unfortunately photographically incorrect, the negative being very thin and without contrast. The resulting picture, whilst showing these defects, nevertheless brings out the peculiar action of the bichloride salt on the material of the degeneration around the muscle fibres. The granular condition of the blebs is well shown, as is the radial arrangement, more minutely described in the text.

Amplification = Gundlach 1/5. Project. ocular No. 4. Camera length 550 mm.

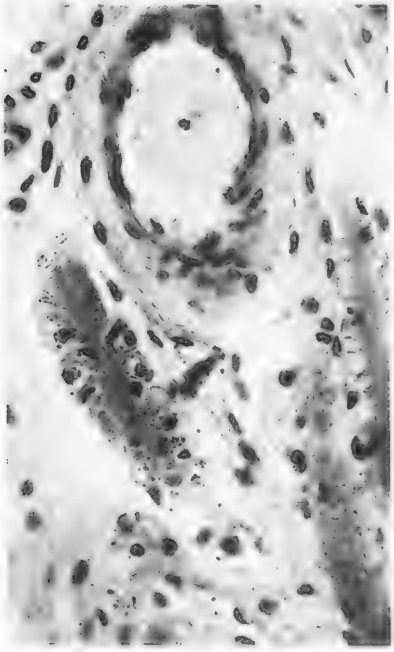


FIG. 9.

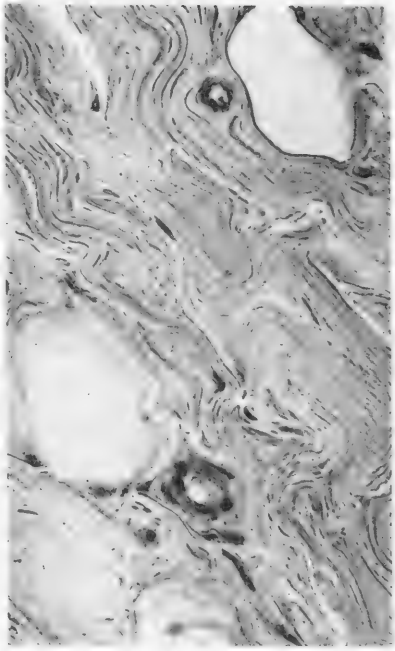


FIG. 10.

FIG. 11.

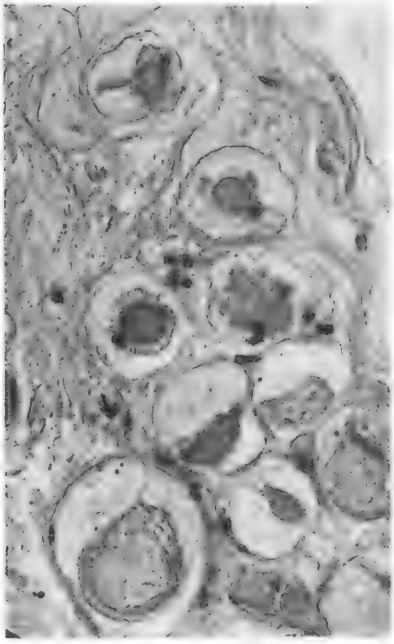
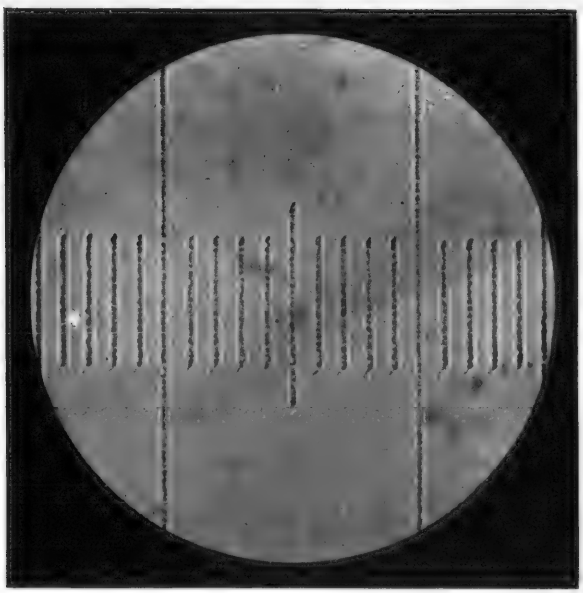


FIG. 12.

PLATE L

Represents a millimetre scale, divided into 0.01. The photo-micrograph was obtained by the combined use of a ⅛ achromatic objective made by Gundlach, Zeiss' projection ocular No. 4 (non-compensative) and a camera length of 550 mm., producing an amplification of ×400, with which combination most of the photo-micrographs illustrating this article were made. When other amplifications have been employed for special purposes, they have been duly noticed in the explanatory pages accompanying the plates.



THE FATE OF THE GIANT CELLS WHICH FORM IN THE ABSORPTION OF COAGULATED BLOOD SERUM IN THE ANTERIOR CHAMBER OF THE RABBIT'S EYE.

By LUDVIG HEKTOEN.

PLATE LI.

In this article it is the purpose to describe briefly the results of some experiments which show that the multinucleated giant cells which form during the absorption of foreign bodies in the tissues may divide into viable cells.

Concerning the extensive literature about giant cells—their formation, function and fate—reference is made to the article in this Journal by the writer entitled "The Fate of Giant Cells in Healing Tuberculous Tissue as observed in a Case of Healing Tuberculous Meningitis,"* in which it is pointed out that giant cells in tuberculous tissue, under certain conditions, undergo progressive changes and separate into small living cells.

THE EXPERIMENTS.

Small pieces of sterile, solidified blood serum, such as is ordinarily used as a culture medium, were inserted under aseptic precautions and cocaine anæsthesia into the anterior chambers of several healthy rabbits' eyes through a small incision in the sclera near the corneal margin. Once a bit of sterile potato was introduced. These operations were skilfully made for me by Dr. Cassius D. Westcott, Instructor in Ophthalmology in Rush Medical College, and I wish to thank him for his kind aid.

After perfecting the technique the operations were followed by but little reaction; in some instances the vessels in the sclera were congested for three or four days, the cornea rather cloudy, the anterior

* *The Journal of Experimental Medicine*, iii (1898), 21.

chamber turbid, and more or less photophobia was apparent. Soon after their implantation a small greyish white rim would become visible around the foreign bodies, which gradually disappeared until, if allowed, only a small, greyish, semi-opaque thickening in the inner surface of the cornea remained. In a few cases the blood serum was gradually dissolved without any accumulation taking place around it, the cornea and anterior chamber remaining perfectly clear. In the case of the bit of potato and when pieces of dry and hard serum were used, the absorption always proceeded slowly and with well-marked anatomical changes.

The animals were killed at intervals of from a few days to six or more weeks after the operation. The eyes were fixed in corrosive sublimate and the piece of cornea and iris, including the site of the foreign body, embedded in paraffin, cut serially, fixed on the slide and stained with hematoxylin and eosin, van Gieson's stain, etc.

THE HISTOLOGICAL EXAMINATION.

Comparatively slight attention was given the study of the early changes. Suffice it to say that the emigration of leucocytes from the vessels of the iris took place to a limited extent only, at least in the cases in which bits of solid blood serum are concerned. The leucocytes present were of the polymorphonuclear variety, and they appeared to become loaded early with granules of serum, after which some of them returned to the iris, in which a few collections of such cells were found. In the later stages of the resorptive process leucocytes were not observed.

The new cells produced come almost wholly, it would seem, from the layer of polyhedral cells clothing the inner surface of the posterior elastic lamina (Descemet's membrane) and the anterior surface of the iris. The posterior lamina itself generally remains quite intact. The new cells, which are round, oval or polyhedral, accumulate around the foreign body and penetrate into its interior, generally breaking it up, so that there is formed a mass of cells which adheres to the elastic layer and encloses the granular detritus of the coagulated serum. Most of the cells take up granules into the interior of their cytoplasm, often to such an extent that the nucleus and the entire cell appear as

if buried beneath the granular mass. The serum granules, whether free or intracellular, are easily recognizable because of their brownish or golden yellow color (derivatives of hæmoglobin pigment).

During this time there are formed a small number of multinucleated giant cells or plasmodial masses, which appear, as far as these observations show, to result from fusion of many small cells. These giant cells do not differ in form and in their general characteristics from those ordinarily observed in connection with the absorption of sterile foreign bodies elsewhere. Their size and the number of nuclei, which are scattered uniformly throughout the body of the cell, vary considerably. In the earlier stages the cells are loaded with serum granules and are of circular outline (Plate LI, Fig. 1); later when the foreign body has been largely removed the giant cells become oval or oblong, due, it would seem, to the pressure of the new fibres that in the meantime have formed around them (Plate LI, Fig. 3).

The granules of solid blood serum without and within the cells gradually disappear, largely, it would seem, by means of a process of intracellular digestion or solution; in some of the giant cells as well as in the smaller cells occur vacuoles in the cytoplasm containing granules; some of the serum, but not much, is also transported into the iris by leucocytes.

Simultaneously with the destruction or removal of the foreign granules the uninuclear and multinuclear cells begin to show further and progressive changes that ultimately lead to the formation of a layer of tissue which much resembles the cornea in structure. These changes begin first and are farthest advanced at the periphery of the cell mass, extending little by little throughout the central parts.

The changes consist in the production of a fibrillated intercellular substance, or stroma, while at the same time the cells become spindle-shaped and elongated, the nuclei more slender and fusiform. All the stages in this transformation of embryonal cells to mature tissue, during which the granules of serum are destroyed, can be quite readily traced. In the later stages the fibrillæ of the new stroma are gathered together into interlacing bundles of considerable thickness (Plate LI, Figs. 2 and 3), the general course of which is parallel with

that of the corneal lamellæ, from which they are separated by the intact posterior elastic lamina. While the course and arrangement of these new fibres are identical with those of the cornea, yet the new lamellæ are not so thick and densely interwoven as those in the cornea and they stain more deeply with eosin. This new tissue, the free surface of which becomes covered by a distinct endothelial lining, may finally appear as a mere rounded or pyramidal-shaped thickening on the posterior surface of Descemet's membrane (Plate LI, Fig. 4), or it may unite the cornea with the iris, especially if the foreign body found a resting place in the irido-corneal angle.

Lying in the spaces of the new tissue, sometimes apparently upon its fibres, are small cells like connective tissue cells, occasionally cells containing granules as well as free heaps of serum detritus and, in the stages not too far advanced, multinucleated giant cells in the process of separation into uninuclear cells.

It has already been stated that in the earlier stages the giant cells are more or less circular and the margins smooth and even. As the fibrillated stroma forms about them they may in many cases become oval and even distinctly oblong (Plate LI, Fig. 3), simultaneously with the disappearance of the serum, the long diameter corresponding to the direction of the fibres of the stroma. At this time they are usually entirely, or almost entirely, free from granules of serum; occasionally there may be vacuoles containing granules in the protoplasm. It should be emphasized that there are no signs of degeneration in the giant cells; the cytoplasm is reddish when stained by eosin and sometimes finely granular, but often it appears dense and homogeneous; the nuclei stain perfectly, and in one instance a dividing nucleus in the daughter star stage was observed in a giant cell.

As the serum disappears more or less distinct lines of cleavage become visible in the giant cells, at first most marked at the periphery; the margin is not smooth and even, but is fringed by the spindle-shaped projections of partly separated cells which seem to have a tendency to arrange themselves parallel with the fibres of the stroma (Plate LI, Fig. 3). Single uninuclear cells exactly similar to these partly detached masses are occasionally found about the giant

cells. In some giant cells, especially those encountered in the older and firmer parts, there are lines of separation throughout their entire extent (Plate LI, Fig. 3), running in such a manner that single nuclei surrounded by more or less cytoplasm are completely or incompletely mapped out; it is noteworthy that in these cells the lines of cleavage map out more or less distinctly spindle-shaped cells, the long axes of which correspond in direction to the fibres of the stroma. Occasionally the lines separating a nucleated mass are so broad and well marked that it appears as though a distinct cell were lying in a vacuole; the cytoplasm of such giant cells especially seems firm and condensed; the splitting up apparently takes place more slowly, and the impression is received that this may all be caused by the pressure exerted upon the giant cell by the fibrillated stroma in which it is enclosed, or that the cytoplasm is being transformed into the same sort of substance as the fibres of the stroma. The separation of small cells nevertheless continues, because in the older tissue are found small giant cells containing a few—three or four—nuclei and embedded in a dense capsule of nucleated fibrous tissue, without any traceable evidences of nuclear disintegration or of destruction of the giant cells by phagocytes.

When the serum is absorbed rapidly and the giant cells lie in the broad and loose meshes of the new-formed fibrillated tissue, the lines of cleavage may be quite complete, so that single nuclei, surrounded by an irregularly shaped bit of cytoplasm, are split off and imperfect giant cells result, which are surrounded by uninuclear small cells that fit the depressions and elevations on the remnant of the subdividing plasmodial mass. Uninuclear cells thus produced present no signs of necrobiosis and can be traced by means of transition stages into the elongated and flattened cells above described as lying in the new-formed tissue. Nuclear and protoplasmic detritus, as well as evidences of immigration of small cells or leucocytes into giant cells, are not to be observed.

CONCLUSION.

The conclusion seems warranted that the giant cells formed in the absorption of coagulated blood serum inserted into the anterior cham-

ber of the rabbit's eye subdivide again into uninuclear small cells that take part with other new-formed cells derived from the lining of this space to form a densely fibrillated mass of tissue that resembles quite closely the cornea in its structure.

This demonstration materially strengthens the opinion expressed by the writer in his previous article in this Journal,* that the giant cells in healing non-degenerated tuberculous tissue may separate into small living cells, and that the giant cells of tuberculosis are not necrobiotic elements from the very moment and from the very mode of their formation, as has been the general teaching, especially in Germany.

DESCRIPTION OF PLATE LI.

Fig. 1.—Recent giant cell with rounded and smooth margin filled with granules of serum so that the nuclei are covered over.

Photo-micrograph, 500 diameters, of eosin and hæmatoxylin specimen.

Fig. 2.—Photo-micrograph. Separating giant cell lying in the meshes of new fibrous tissue and surrounded by cells and by a cell mass which appear to have been sundered from the larger mass; distinct lines of cleavage are seen in the smaller as well as in the larger mass, so that small uninuclear cells may be said to be separating.

Same magnification and staining as in Fig. 1.

Fig. 3.—Oblong giant cell with a dense protoplasm in which run distinct lines that subdivide the multinucleated mass into more or less well-marked, small, uninuclear cells. In the centre is a part of a cell lying in a distinct vacuole. From a late stage. To the left of the giant cell and the surrounding thick fibres runs the intact posterior elastic lamina, and to the right is the substance of the iris.

Photo-micrograph with same magnification and staining as in Fig. 1.

Fig. 4.—Fusiform thickening of posterior surface of cornea, due to the formation of a densely fibrillated mass of new tissue after complete absorption of the blood serum. The posterior surface of the new-formed mass is clothed with a single layer of endothelial cells which is continuous with the lining of the posterior surface of Descemet's membrane. Close inspection will show the intact posterior elastic lamina running between the new tissue and the cornea.

Photo-micrograph, 100 diameters, of eosin and hæmatoxylin specimen.

* Loc. cit.

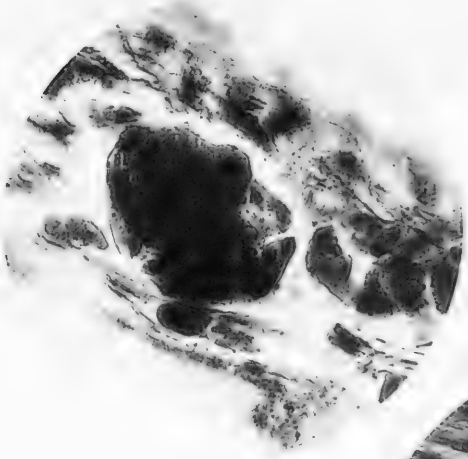


FIG. 1.

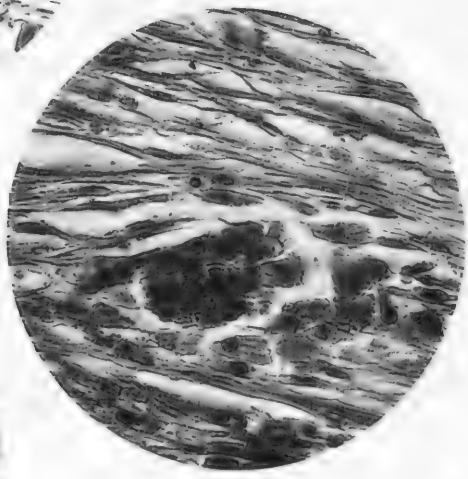


FIG. 2.

FIG. 3.

FIG. 4.

THE HELIOTYPE PRINTING CO. BOSTON

REGENERATION OF NERVE FIBRES IN THE CENTRAL NERVOUS SYSTEM.

By W. L. WORCESTER, M. D., Assistant Physician and Pathologist, Danvers Lunatic Hospital.*

Plate LII.

The tendency of the peripheral nerves to regeneration and restoration of function after division is well known. There would seem to be no reason, *a priori*, why the processes of the neurones within the central nervous system, so similar to the peripheral fibres in structure and function, should not conduct themselves in a like manner; but it is notorious that such is not, at least commonly, the case. Whether the interruption of continuity is due to traumatism or to disease, the rule seems to be that there is no attempt at repair of damage.

In view of this well known fact, it seemed to me that a case coming under my observation, in which there is, in my judgment, beyond reasonable doubt, a growth from interrupted fibres in the central nervous system, deserves to be placed on record. I have been able to find in the literature of the subject but little lending countenance to the belief that such regeneration ever takes place.

Brown-Séquard (1) reported in 1851 a fairly complete restoration of sensibility and partial restoration of movement in a guinea-pig after section of the spinal cord. Microscopical examination of the cord, by himself and Laboulbène, showed very numerous medullated fibres extending through the cicatrix.

Eichhorst and Naunyn (2) in 1874, experimenting on new-born puppies, in which they destroyed the spinal cord by pressure with a blunt instrument without opening the dura, found evidence of restoration of function in some cases, beginning from the third to the fifth week after operation. In from seven to nine weeks the puppies were able to walk on all fours ten or fifteen feet. The movements were very awkward and

* Read at the meeting of the American Neurological Association, New York, May 28, 1898.

ataxic. These observers thought they got some evidence of restoration of sensibility, but admitted that it was very slight. No further improvement took place; on the contrary, there was generally retrogression after ten or twelve weeks. The histological examination showed in the cicatricial tissue connecting the segments of the cord small numbers of medullated nerve fibres, resembling rather those of the peripheral nerves than of the spinal cord.

Schiefferdecker (3) criticized the foregoing paper severely. Although he did not dispute the presence of newly formed nerve fibres in the cicatrix, he believed that the authors had mistaken reflex for voluntary movements, and denied that there was any restoration of function. His own experiments made on dogs several months old all gave entirely negative results. In consequence of Schiefferdecker's criticisms, Eichhorst (4) took up the subject again. He operated as before on newborn puppies, but isolated the cord and lifted it out of the canal, so that there could be no question of its complete division. He obtained a positive result in only one of three puppies. This one began to move the hind legs at the end of the fourth week; began to walk on the 26th day, and on the 35th day walked continuously a distance of about 180 feet. No evidence of return of sensibility could be found. The animal was killed at that time, and the continuity of the cord was found to be restored by a tissue containing very numerous nerve fibres.

Sgobbo (5) and Marinesco (6) in their experiments by division of the spinal cord both failed to get any evidence, whether functional or anatomical, of regeneration. Popoff (7), in studying the lesions of multiple sclerosis, satisfied himself that the naked axis cylinders found in the lesions were not, as has been generally supposed, remnants of the original constituents of the cord, but, in many instances at least, newly-formed fibres. He claims to have seen the division of a single axis cylinder into a number of fine fibres.

Stroebe (8), experimenting by section of the spinal cord in rabbits, found what he considered unquestionable evidence of growth of nerve fibres into the scar from the posterior nerve roots. In the anterior and lateral columns he found fine medullated fibres in the zone of degeneration, often presenting a contorted, confused course, which he judged to be newly formed. They never passed the line of incision. He concludes that there is an effort at regeneration, but that it never comes to a restoration of continuity in the nervous structure.

Borst and Nicolaier (9), in a case of fracture of the vertebral column in the lower thoracic region in which the patient had survived the injury for three years, found numerous medullated fibres, resembling those of

the peripheral nerves, in the region of both the posterior and anterior nerve roots, from which he considers them to be outgrowths, comparing them to the amputation neuromata.

The case to which I ask attention is that of a married woman, aged 41 at the time of her admission to the Danvers Lunatic Hospital, September 28, 1885. Very little information was furnished in regard to her antecedents beyond the fact that she had suffered from repeated attacks of maniacal excitement. At the time of her admission she was much demented; there is no history of any subsequent maniacal outbreak. During her stay in the hospital she had two paretic attacks, both affecting the left side, leaving permanent impairment of motion and sensation, with paræsthesia, which gave rise to delusions that her left side was maltreated in various ways. After the second attack she was confined to her bed until her death, nearly two years afterward.

When she came under my observation in the spring of 1895 there was very marked impairment both of motion and of sensation in all its modes in the entire left side with the exception of the face. She complained of pain in that side, which she attributed to "people throwing things on her." On September 21, 1895, she had an epileptiform convulsion from which she never rallied, dying September 27.

At the autopsy both right corpora quadrigemina were found to be much smaller than normal and of a yellowish brown color. The bodies, together with the pons and medulla oblongata, were hardened in a solution of bichromate of potassium and cut in serial sections for the purpose of studying the secondary degenerations. It then appeared that there was also a large focus of softening in the right side of the pons, involving the pyramidal tract extensively and encroaching on the lemniscus. With the secondary changes from these lesions I am not at present concerned.

On examination of the sections the first-mentioned lesion was found to involve the whole of the right corpora quadrigemina, passing a little beyond the median line above the aqueduct and extending downward laterally to the border of the pes pedunculi. It did not

extend below the central gray matter surrounding the aqueduct (see Plate LII, Fig. 1). Microscopically it was seen to consist of a dense felt-work of neuroglia fibres, rather sparsely supplied with vessels.

In the anterior corpus quadrigeminum, approximately in the centre of the diseased area, I found a group of medullated nerve fibres (Plate LII, Fig. 1, *a*) which I at first supposed to be a normal tract which had escaped the destruction that had befallen the surrounding tissue. On tracing it through successive sections, however, I found that the number of fibres diminished in each direction, and that it finally disappeared at each end, without any apparent connection, either proximally or distally, with any normal tissue. On comparing it with the corresponding region on the opposite side, no group of fibres could be found presenting an arrangement at all resembling it. The appearance at the largest portion of the tract can be seen in Fig. 2; near its proximal and distal extremities in Figs. 3 and 4 respectively.

The tract consists of a number of bundles of fibres, showing an irregular, convoluted arrangement, without any apparent order or system. Careful examination of specimens, stained in various ways, failed to reveal any nerve cells in or about this area. In a number of sections, however, small bundles of fibres could be seen extending between it and the zone of white substance surrounding the red nucleus (Plate LII, Fig. 5, *b*). This was the only connection that I could trace between it and normal tissue and must, I think, be the origin of the group in question.

Such are the facts in the case. As to the interpretation to be put upon them, I can see no reasonable hypothesis other than that the fibres in question are outgrowths from neurones that were interrupted in their course by the lesion. That they are not simply a remnant of the original structure seems certain from the fact that nothing at all resembling them can be found in normal specimens from the same region.

Moreover, it will, I think, be evident to any one who studies the specimens that it is entirely improbable that such a tract could be preserved in the very centre of the focus of disease. The appearance

of these irregular, contorted bundles of fibres, so strikingly similar to that of an amputation neuroma, is also, to my mind, strongly confirmatory of this view.

If this is the correct interpretation of the facts, it would appear that nerve fibres in the central nervous system, after solution of continuity, may be capable of growth. Of course, in this case, there is no question of regeneration in the sense of restoration of the original structure or function. In the present state of our knowledge this finding must be considered rather as a curiosity than a matter of practical importance.

REFERENCES.

1. Brown-Séquard.—*Gaz. méd. de Paris*, 1851.
2. Eichhorst and Naunyn.—*Arch. f. exper. Pathol. u. Pharmakol.*, ii, 1874.
3. Schiefferdecker.—Virchow's *Archiv*, lxvii.
4. Eichhorst.—*Zeitschr. f. klin. Med.*, i, 284.
5. Sgobbo.—*La Psichiatria*, 1890.
6. Marinesco.—*Centralbl. f. Nervenheilk.*, Oct., 1894.
7. Popoff.—*Neurol. Centralbl.*, May 1, 1894.
8. Stroebe.—Ziegler's *Beiträge z. path. Anat.*, xv, 1895.
9. Borst.—Berichte über Arbeiten aus dem Path.-anat. Institut der Universität Würzburg, 1. Folge, p. 34. Würzburg, 1897.

DESCRIPTION OF PLATE LII.

Fig. 1.—Section through corpora quadrigemina, × 2. Newly formed fibres at *a*.

Fig. 2.—Fibres at *a* in preceding figure, × 40.

Fig. 3.—Newly formed fibres near proximal end, × 40.

Fig. 4.—Same, near distal end, × 40.

Fig. 5.—Same, thickest portion of tract. Bundle of fibres connecting it with tegmentum at *b*.

FIG. 1.

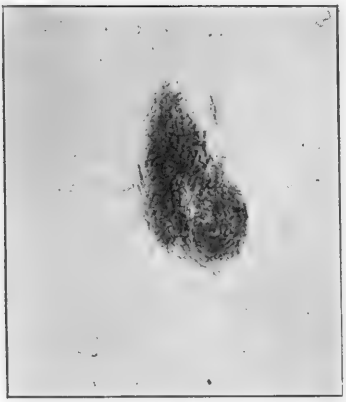


FIG. 3.

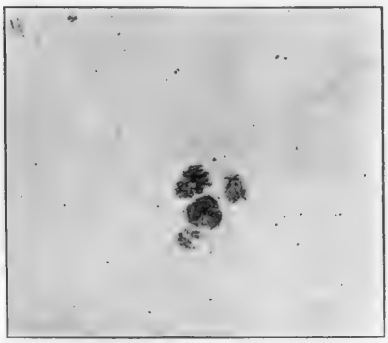


FIG. 4.

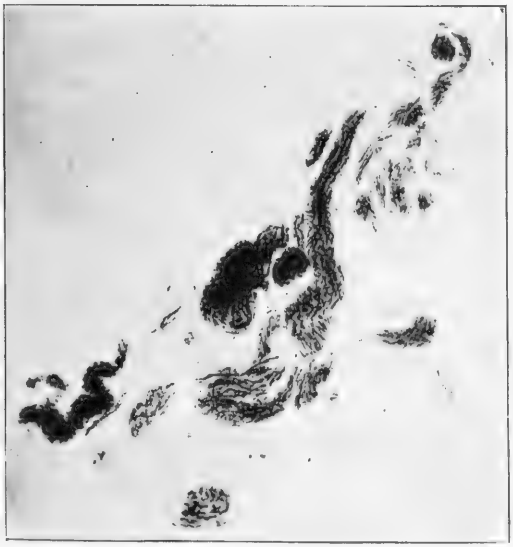


FIG. 2.

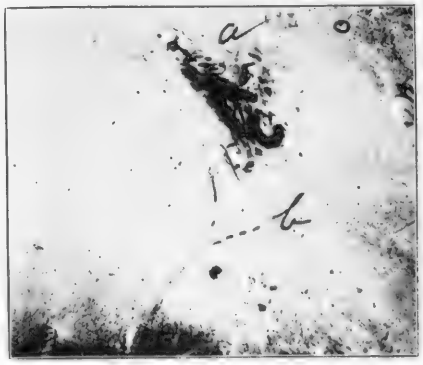


FIG. 5.

EXPERIMENTAL FAT NECROSIS: THE ACTION OF PANCREATIC TISSUE UPON SUBCUTANEOUS ADIPOSE TISSUE.

By HERBERT U. WILLIAMS, M. D.

(*From the Pathological Laboratory of the University of Buffalo.*)

PLATE LIII.

In a paper by the writer published in 1897* an account was given of some experiments which sought to discover the cause of the "fat necrosis" so often associated with acute disease of the pancreas. Since fat necrosis consists of a decomposition of the neutral oils contained in fat cells, the attempt was made to discover whether that change could be accomplished by the pancreatic juice, which has a ferment capable of decomposing neutral oils. To this end a ligature was placed about the gastro-splenic portion of the pancreas near the duodenum. In some cases as many as possible of the veins leaving the pancreas were tied and the substance of the gland was lacerated with a sharp hook. The object of the operation was to prevent the outflow of the pancreatic juice into the duodenum and to permit it to act upon the adjacent adipose tissues. The operation was performed upon twenty animals, mostly cats. In five cases well-marked fat necrosis ensued. There were three indecisive and twelve negative results. In three of the five cases where positive findings were obtained an accidental micrococcus infection was associated. It was not, therefore, considered justifiable to attribute the fat necrosis to the action of the pancreatic juice alone.

In order to discover whether bacteria played any important role in the production of fat necrosis, operations similar to those described have since been undertaken, where infection with pyogenic bacteria was brought about intentionally.

* *Bost. Med. and Surg. Journ.*, April 15, 1897.

Cat 37. Large, fat. The gastro-splenic portion of the pancreas and some of the veins issuing from it were ligated, the pancreas was lacerated with a sharp hook, and its surface was smeared with a quantity of the staphylococcus pyogenes aureus from a fresh culture on agar. Upon the following morning the cat was found dead. The autopsy showed beginning peritonitis and widely spread fat necrosis in the adipose tissue near the pancreas and in the omentum, in the form of a thin, flat layer, including the most superficial cells just below the peritoneum. The staphylococcus pyogenes aureus was recovered in cultures from the peritoneum, liver and spleen. Examination of the other viscera yielded nothing noteworthy. Microscopic sections showed well-marked beginning pancreatitis.

Cat 44. Medium sized, fairly well nourished. The gastro-splenic portion of the pancreas was ligated close to the duodenum, also several veins leaving it; it was lacerated with a sharp hook, and the surface was smeared with the streptococcus pyogenes from a culture made recently from pus on agar. On the fourth day the animal was found dead. Autopsy revealed peritonitis, especially near the pancreas. A part of the tail of the pancreas was disorganized and necrotic. There were numerous evidences of fat necrosis in the retro-peritoneal fat of the pancreatic region and in the omentum, and a few scattered spots appeared in the mesentery and meso-rectum. Some of the areas were large and flat, others were localized. The lungs showed pneumonia and fibrinous pleurisy. Smears from the peritoneum yielded a streptococcus and a large bacillus. The streptococcus alone was recovered in cultures. The evidences of acute pancreatitis were very marked in microscopic sections.

Cat 45. Large, well nourished. The gastro-splenic part of the pancreas was ligated; there were no laceration and no ligation of the veins, but the surface was smeared with the staphylococcus pyogenes aureus. The animal remaining healthy in appearance was killed at the end of a week. A single small area of fat necrosis was found close to the pancreas. Localized opacities due to thickening of the peritoneum were seen, but there was no general peritonitis. Pancreatitis was well marked.

The proportion of positive results was larger from these than from the first series of operations, but the number of cases was too small to permit one to draw far-reaching conclusions. The extensive fat necrosis in Cats 37 and 44 seemed as likely to have been due to the laceration of the pancreas as to the introduction of the pyogenic cocci, though the latter may have served to hasten or intensify the result.

It will be noticed that in Cat 37 extensive fat necrosis occurred within twenty-four hours after the operation on the pancreas. As following this line of procedure did not seem likely to lead to a solution of the problem under consideration, it was abandoned. Furthermore, on account of the instability of the fat-splitting ferment, injections of pancreatic extracts appeared to offer an unpromising prospect.

In an experiment described in the article already mentioned a large piece of fresh cat's pancreas was inserted into the abdominal cavity of another cat, with the view of observing the effects of its ferments on the peritoneal adipose tissues. Although fat necrosis followed, an accidental infection of the peritoneum and of the animal's own pancreas also took place and nullified the results. Owing to the danger of thus confusing the possible effects of disease of the animal's own pancreas with those of the pancreas artificially introduced, the peritoneal adipose tissue was deemed an unsuitable location, and efforts were then made to insert pieces of pancreas into the subcutaneous or intermuscular fat. As before, the cat was the animal selected. Numerous attempts to cultivate bacteria from the fresh cat's pancreas planted upon agar and kept in the incubator found it to be sterile in each case. In most of the following experiments the pancreas used was tested for its fat-splitting power, always with positive results. The locations chosen for the operations were the inguinal regions and a point a little below the sternum. The skin of the vicinity was shaved and washed with alcohol and an antiseptic solution. The instruments and silk used were sterilized with heat. The pancreas was taken fresh and with great care from the abdomen of another cat. It was inserted into the adipose tissues through a small incision, and was held in position by a silk suture, not passing through it, but uniting the edges of the tissues at the bottom of the wound. The superficial incision was closed separately with silk and collodion.

Cat 27. Thin. A piece of fresh pancreas from another cat was introduced through a small incision into the subcutaneous adipose tissue of the right inguinal region. After four days a large abscess had resulted, undoubtedly from accidental infection. The animal was killed and the abscess removed entire for histological examination. Besides

the ordinary changes of suppuration, the walls of the abscess showed areas of fat cells presenting a reddish blue stain with hæmatoxylin and eosin and contents having the homogeneous appearance seen in undoubted fat necrosis. Some cells exhibited large needle-shaped crystals. Owing to the occurrence of suppuration the result was considered indecisive, but as probably indicating fat necrosis.

Cat 30. Healthy, young animal, quite fat. Portions of fresh pancreas from another cat were introduced into the subcutaneous adipose tissue of the right inguinal region and into the adipose tissue below the sternum behind the linea alba. After four days the points of introduction had become immense abscesses. The animal was killed and both abscesses were removed entire. The histological examination gave the same result as in the last case.

Cat 33. Healthy young cat, well nourished. Three localities were utilized for the introduction of cat's pancreas into the adipose tissues: the middle line below the sternum, the middle line above the symphysis pubis, and the right inguinal region. After five days abscess formation had begun, so that the animal was killed and the abscesses were excised. The histological examination detected no fat necrosis.

Cat 35. Large, well nourished. A piece of fresh cat's pancreas was introduced below the sternum in the same manner and with the same precautions as previously, except that a portion of adipose tissue was excised to make room for the piece of pancreas. After three days an immense abscess had resulted. The animal was killed and the abscess removed. The examination of sections with the microscope gave the changes to be expected for suppuration, with alterations in the fat cells similar to those already mentioned for cats 27 and 30.

The experiments just described seemed to show the futility of continuing along the lines followed in them. Although cultures were not made from the abscesses encountered, it was sufficiently evident that they proceeded from infection. In cases related hereafter, where identical results were obtained, the micro-organisms causing the suppuration were demonstrated. (Cats 43 and 50.) It was impossible to determine how far the changes in the tissues were to be attributed to bacteria, and how far to the working of the pancreatic ferments. As has been remarked, the infection probably took place from organisms on the skin, as the pancreas was removed with such care as to render it above suspicion. The writer has been led to

suspect that the pancreas may be possessed of irritating properties which make the tissues in contact with it extremely liable to infection.

In order to observe the effect of perfectly sterile pancreas on the tissues the following technique was devised, which can be recommended when it is desired to insert solid substances hypodermically. A cannula of glass of the size and shape represented in the accompanying figure was made from ordinary glass tubing. It was sterilized in a Petri dish, and the portion of pancreas to be used was placed in the large end, with a bit of sterile black silk. The pancreas and silk were forced along to the small end with a stiff platinum wire. The skin having been shaved, cleaned and rendered aseptic as far as possible, a small incision was made through it. The adipose tissues underlying it were nicked. The small end of the cannula was then forced into the adipose tissue three or four centimetres. The pancreas and bit of silk were pushed out of it by the wire, depositing them in the desired locality, after the withdrawal of the cannula. The surface wound was closed with one or two sutures. The success of this plan was very gratifying. In seventeen experiments made according to it upon nine cats, eleven were satisfactory in excluding bacteria, while infection occurred in but four instances and two experiments in another animal indicated doubtful infection. The black silk served admirably to identify the point of introduction.

Cat 38. Large, fat. Pieces of fresh pancreas one to two millimetres in diameter were inserted into the adipose tissues just below the sternum and in the right inguinal region, after the manner just described. On the twelfth day there had been no evidence of inflammation, and the wounds in the skin were nearly closed. The animal was killed. The points where the pancreas had been inserted were at once located by means of the black silk. Agar tubes were inoculated from both, and placed in the incubator. These tubes remained sterile. Serial sections of both regions were cut; those from below the sternum were negative; in the inguinal region a small area of fat necrosis was found.

Cat 39. Large, fat, not very vigorous. Pieces of fresh cat's pancreas

one to two millimetres in diameter were inserted through the sterile cannula into the adipose tissues of the right groin and below the sternum. The wounds of the skin healed rapidly and without incident. The animal was killed three weeks after the operation. Cultures were made from both points, which remained sterile. The examination of sections showed no fat necrosis.

Cat 40. Small, thin. A small piece of fresh cat's pancreas was placed in the fat tissue of the right groin through the cannula. The wound remained healthy. The cat was killed at the end of six days. Cultures from the locality remained sterile. Examination of sections did not demonstrate fat necrosis.

Cat 42. Small, not fat. By means of the sterile cannula small bits of fresh cat's pancreas were introduced into the adipose tissue in the sternal and left inguinal regions. After the lapse of seventeen days the points in question were examined. A positive result was obtained in the left groin, where a small spot of fat necrosis was found. Agar tubes from this area showed no growth. No bacteria could be found in sections stained by the Gram-Weigert method and with methylene-blue.

Cat 43. Large, fat. Through a glass cannula, bits of fresh cat's pancreas were inserted in the adipose tissues in three places: the right and left inguinal regions and the region below the sternum. At the end of three days there was much swelling and slight fluctuation. The cat was killed. Smears were made and agar tubes inoculated from all three localities, and the spots were excised. Suppuration was evident in all, and gas formation, as well, below the sternum. The pancreas taken in this case was from the same source as that used for Cat 42 and had produced no growth on agar. As Cat 42 had shown no suppuration, the source of infection in Cat 43 was probably the skin. The cause of the suppuration proved to be an undetermined micrococcus. Examination of sections showed probable fat necrosis in both inguinal regions, but none in the locality below the sternum.

Cat 47. Small, sickly, not fat. Pieces of fresh pancreas of a young cat were placed in the right and left inguinal adipose tissues through the glass cannula. The cat died unexpectedly two nights later. Autopsy showed the cause of death to be bronchitis and broncho-pneumonia, probably having no relation with the operation. The edges of the wounds had parted slightly, but there was no swelling in the neighborhood, nor any of the macroscopic evidences of suppuration. Cultures from both sides yielded a small bacillus, resembling B. coli communis. The weather during this period was excessively hot, and the results of the cultures were not deemed positive evidence of ante-mortem infec-

tion of the points of introduction. On both sides, in the neighborhood of the inserted bits of pancreas and for two to three millimetres in the track taken by the cannula, the adipose tissue had a strikingly white, opaque, cheesy appearance. Examination with the microscope, fresh and in sections, demonstrated the histology of fat necrosis.

Cat 48. Large, moderately fat. The fresh pancreas of a half-grown kitten was used. Small bits were inserted into the adipose tissues of the right and left inguinal regions through the glass cannula. At the end of five days the cat, appearing healthy, was killed. The regions where the pancreas had been placed were not swollen; but the adipose tissues for two or three millimetres around presented a white, cheesy appearance. Examination of the cheesy matter fresh, and of the region in sections, showed the characters of fat necrosis. Smears and sections stained for bacteria and culture tests all gave negative results.

Cat 49. Medium sized. Bits of fresh pancreas from a full-grown cat were introduced by means of a sterile glass cannula into the adipose tissues of both inguinal regions. After eight days, appearing healthy, the cat was killed. The skin wounds were in good condition. There was slight swelling where the pancreas had been placed, but no suppuration. There were, however, numerous minute, curd-like, white spots on the edges of the adjacent adipose tissues. Agar tubes inoculated from the region remained sterile. Smears stained for bacteria gave negative results, as did the subsequent examination of sections of the tissue for bacteria. Inspection of the curd-like masses and study of sections of the tissues gave the histology of fat necrosis.

Cat 50. Small. Received a bit of fresh cat's pancreas in the inguinal region through the sterile glass cannula. At the end of eight days swelling and fluctuation indicated pus formation. The pus contained an undetermined diplococcus. Nevertheless, the histological characteristics of fat necrosis were demonstrated in fresh preparations and in hardened and stained sections.

Summary.—Thirteen cats were used for the subcutaneous introduction of pancreas into the adipose tissues in twenty-four places. At nine points fat necrosis ensued; in nine there was no fat necrosis; in six the result was doubtful. The doubtful results were in cases in which suppuration complicated the issue. At eleven points there was no infection, two were doubtful, and eleven became infected. Selecting the eleven in which there was no infection and the two classed as doubtful, where infection was probably post-mortem, in all

thirteen, we find eight points at which fat necrosis occurred and five from which it was absent.

General description of the lesions at the site of introduction of the pancreas.—In those cases which resulted favorably and where the autopsies were performed only a few days after the subcutaneous insertion of the pancreas, the adipose tissues in that vicinity, which was identified by the piece of black silk, acquired a white, opaque and cheesy character.

The opacity was found to reside in certain fat cells; while adjacent fat cells presented a normal appearance. The altered cells exhibited a brown tinge, and their contents consisted of minute linear crystals. Ring-shaped plates were seldom seen, while they were common in the necrosis obtained in the cat after operations on the pancreas. Fragments of similar opaque material were seen which appeared to have arisen from the breaking up of such altered cells. Loose crystals and granules were also abundant.

The amount of material was too small to permit the application of micro-chemical tests in a perfectly satisfactory manner in all instances. When they could be employed the reactions were those characteristic for fat necrosis, including sometimes the demonstration of calcium salts.

The tissues were hardened in alcohol or in formaldehyde and embedded in celloidin. Sections stained with hæmatoxylin and eosin gave satisfactory pictures. The region where the pancreas had been placed was readily discovered by means of the black silk which had been introduced with the piece of pancreas. The pancreas itself could not be identified with certainty. Fragments of disorganized tissue associated with the bits of silk, staining with eosin, not stained by the Gram-Weigert method, and exhibiting neither nuclei nor definite structure, were supposed to represent the pieces of pancreas.

The altered fat cells were found on the surface of the cavity containing the silk and these fragments of tissue (Plate LIII). Their contents stained intensely, partly with hæmatoxylin, partly with eosin. The contents consisted in some cases of homogeneous material, in some cases of needle-shaped or fine feathery crystals, plainly vis-

ible in balsam preparations. They often contained granules, which stained intensely with hæmatoxylin, of very variable size, usually round, but sometimes quite irregular in form. Similar granules occurred between the cells. Some of these granules may have owed their intense stain to the presence of calcium salts. Some of them appeared to have arisen from the breaking up of nuclei. Nuclei belonging to the necrotic fat cells could not be discovered. The altered fat cells were of normal size or slightly enlarged at most. Necrotic cells were found, which were almost completely disintegrated, and with them loose crystals and fine granular material.

Between and around the necrotic fat cells polynuclear leucocytes appeared, usually so as to make an extensive accumulation, though sometimes in relatively small numbers. The interstices of the neighboring unaltered adipose tissues also contained leucocytes, which, with the granules above mentioned, occasionally constituted a well-marked zone around the region of operation. Larger cells with undivided nuclei, probably endothelial, were visible in smaller numbers, especially around the fibres of silk. Fragmentation of nuclei was often seen. A fine reticulum, stained by the Gram-Weigert method, which was observed sometimes in the area of most intense necrosis, suggested the formation of fibrin. In no case was there marked evidence of hæmorrhages. Where the time after operation was longest (12 days in Cat 38, 17 days in Cat 42) the silk and area of necrotic cells were enclosed in richly cellular, new-formed fibrous tissue. In the same cases there was less accumulation of leucocytes than in the more recent ones.

It will be seen that in all essential respects the histological characters of subcutaneous adipose tissues which are subjected to the direct action of the excised pancreas may be identical with the peritoneal fat necroses observed after ligation, laceration, and other operations on the pancreas. The probability, therefore, becomes very strong that fat necrosis may be produced by the unaided action of the ferments of the pancreas.

DESCRIPTION OF PLATE LIII.

Fig. 1.—Cat 47. Left inguinal adipose tissue, showing unaltered and necrotic fat cells, accumulation of granules and leucocytes at point of junc-

tion, black silk and dark fragment of tissue close to it, probably pancreas; two days after insertion of pancreas. Hæmatoxylin and eosin. Photo-micrograph by H. M. Hill. Spencer ⅔-inch objective. ×70.

Fig. 2. Cat 42. Part of the area of fat necrosis from the left inguinal region; seventeen days after insertion of pancreas. Photo-micrograph by H. M. Hill. Spencer ⅔-inch objective. ×70.

Fig. 3.—Cat 48. Left inguinal region, showing isolated fragment of adipose tissue, almost wholly necrotic, and leucocytes. Hæmatoxylin and eosin. Low-power water-color sketch by Miss E. M. Porter.

FIG. 1.

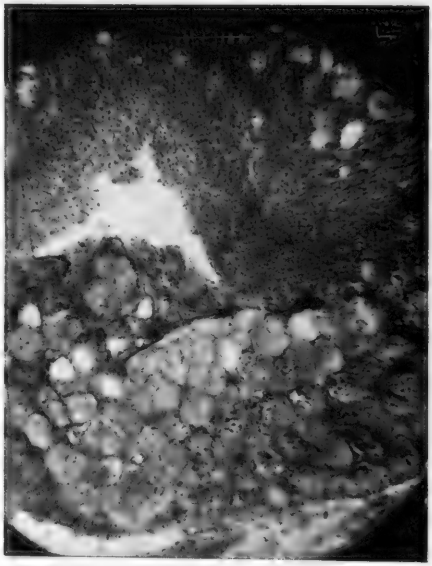


FIG. 2.

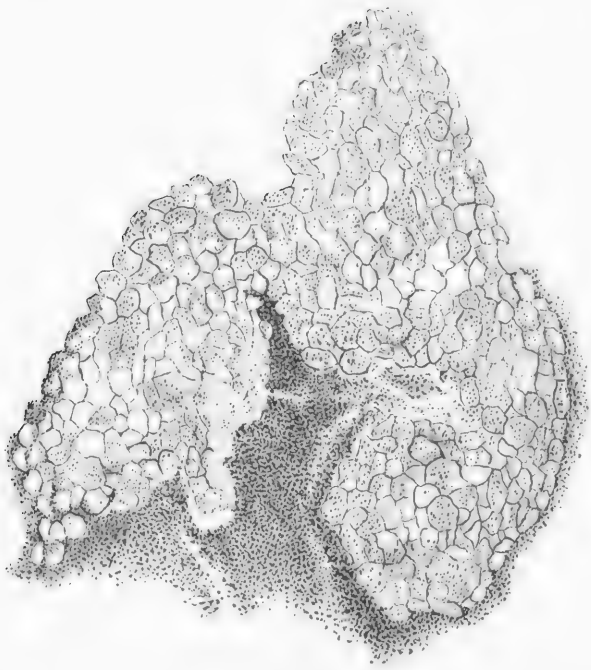


FIG. 3.

A CONTRIBUTION TO THE STUDY OF THE PATHOGENESIS OF THE BACILLUS PYOCYANEUS, WITH SPECIAL REFERENCE TO ITS RELATION TO AN EPIDEMIC OF DYSENTERY.

By AUGUST JEROME LARTIGAU, M. D.

(*From the Bender Hygienic Laboratory, Albany, N. Y.*)

The bacillus pyocyaneus has within recent years attracted attention owing to the results of experimentation with this organism, and also to the publication of a number of isolated instances demonstrating its relation to certain infectious processes in man.

The occasional blue or green staining of surgical dressings has been known for a long time, and the nature of the coloring principle was early ascertained by Fordos,* who was successful in isolating it from such dressings. The cause of the production of the pigment was not, however, discovered until much later, when Gessard,† after a number of unsuccessful attempts by several other investigators, was able to demonstrate that the coloring matter was manufactured by the bacillus pyocyaneus, which he succeeded in growing in pure culture for the first time. Subsequently to Gessard's discovery this bacillus was for some time regarded as a purely saprophytic micro-organism incapable of producing pathological lesions either in animals or in man. Ledderhose‡ was among the first to study experimental infections with the bacillus pyocyaneus, but the present conception of its etiological relations to the production of certain definite pathological processes is due to Charrin's§ admirable researches.

Charrin in his experimental studies of the organism upon animals was able to observe with considerable constancy a marked disposition toward a definite clinical evolution as manifested by the appearance of symptoms which resulted in the presentation of a fairly characteristic clinical entity. To this symptom-complex he gave the name "maladie

* *Compt. rend. Acad. des Sciences*, 1860, li.

† La pyocyanine. Thèse de Paris, 1882.

‡ *Deutsch. Zeitschr. f. Chirurgie*, xxviii, 1888.

§ La maladie pyocyanique. Paris, 1889.

pyocyanique." In rabbits, even when subcutaneous inoculation is resorted to, the systemic symptoms predominate, and it is in this animal that the disease is presented in its most typical form. The symptoms generally noted are diarrhœa, albuminuria, fever, and in the more chronic cases, cachexia and paralyses, which Charrin considers of a perfectly characteristic type, and in which the hind quarters are the ones most frequently involved. The paralysis is essentially spastic in nature. At autopsy the animals show congestion of the mucous membrane of the intestine and usually small hæmorrhages in the various viscera, but more constantly in the intestinal walls. Schäfer,* although he carried on his experimentation with the dog, arrived at results not unlike those obtained by Charrin. Cadéac,† shortly after the publication of Charrin's results, reported a case of spontaneous pyocyaneus infection in the dog. Since this there has been an abundance of experimental evidence which is essentially confirmatory of the conclusions of Charrin, Ledderhose and Schäfer.

The bacillus pyocyaneus has been found in the saliva and in sputum by Pansini‡ and Frick,§ who attribute the green color sometimes observed in sputum largely to this bacterium. It has also been detected in the sweat by Eberth¶ and Audouard.|| Abelous** found it in the stomach, and its isolation from wounds from time to time has been already mentioned. Its presence in suppurating wounds is rather infrequent, as shown by Jakowski,†† who was able to find it in his bacteriological examinations only twice in 200 cases of this nature. This same observer obtained it in almost pure culture from a fistula of the large intestine, and a second time from a fistula of the small intestine. Observations of Koch showed the presence of the bacillus in tuberculous cavities.

Investigators at this time generally believed that its presence and association with pathological conditions were only as a saprophyte. The conception of the bacillus pyocyaneus as an etiological factor, which possibly might at times excite morbid phenomena, was reserved for

* Inaug.-Diss. Berlin, 1891.

† *Comptes rend. de la Soc. de biol.*, 9. s., ii (1890), 41.

‡ Virchow's *Archiv*, cxxii, 424.

§ Ibid., cxvi (1889), 266.

¶ *Medicin. Centralbl.*, 1873, and Virchow's *Archiv*, lxii (1875), 512.

|| *Journal de médecine de l'Ouest*, 1879.

** Thèse de Montpellier. 1888.

†† *Zeitschr. f. Hygiene*, xv, 475.

Gruber,* who obtained the organism from a case of middle-ear disease. The recognition of its power to produce pathological conditions has more recently received elucidation in the observations of a number of clinicians and experimenters.

Martha† reports two cases of purulent otitis media from which pure cultures of the bacillus were obtained. It has likewise been cultivated from many other cases of otorrhœa, especially in children, sometimes in pure culture, at other times in association with other organisms, by Maggiora and Gradenigo,‡ Babes,§ Pes and Gradenigo,¶ Finkelstein,‖ and others. Kossel,** in the bacteriological examination of 52 cases of purulent middle-ear disease, isolated it eight times, and in three of these cases was able to cultivate it from the blood. Charrin†† found it in a case of puerperal fever associated with a few streptococci and also isolated it from a case of chronic mastitis. Blumer‡‡ cultivated the organism in practically pure culture from a case clinically resembling diphtheria; Monnier§§ from the saliva in noma and also from a meningeal exudate. Babes¶¶ reported a localized pyocyaneus infection of the umbilical cord, while H. C. Ernst‖‖ obtained the bacillus from the pericardial exudate during life from a case of tuberculous pericarditis. In addition to these cases of local infection, it has been demonstrated in an ovarian abscess by Barker,*** and the same writer has studied it in diphtheritic inflammation of the œsophagus, broncho-pneumonia, ureteritis, pyelonephritis, and intestinal ulceration, as well as in cases of general infection. In his paper Barker also cites two cases of hepatic abscess reported by Kruse and Pasquale. The micro-organism has been isolated from a number of cases of urinary infection by Jadkewitsch,††† Oettin-

* *Monatschr. f. Ohrenheilk.*, No. 7, 887.
† *Arch. de méd. expér.*, iv (1892), 130.
‡ *Ann. de l'Instit. Pasteur*, t. v.
§ Cornil et Babes. Les Bactéries, p. 486.
¶ *Gazz. med. di Torino.*, xlv (1894), 521.
‖ *Charité-Annalen*, p. 346, 1896.
** *Zeitschr. f. Hygiene*, xvi, 1894.
†† Loc. cit.
‡‡ *Johns Hopkins Hosp. Bull.*, 1895.
§§ *Gaz. méd.*, Nantes, 1895.
¶¶ Loc. cit.
‖‖ *Am. Jour. Med. Sci.*, cvi (1893), 396.
*** *Jour. Amer. Med. Assn.*, 1897.
††† Baumgarten's *Jahresbericht*, p. 355, 1890.

ger,* B. Motz,† and Le Noir,‡ who obtained it either in pure cultures or associated with other bacteria.

It is evident from the data at hand that the bacillus pyocyaneus is capable of causing a whole series of localized lesions, acute or chronic, of very different sorts, being in this respect not unlike the common pus cocci. The causative relation of this organism to such a large number of processes whose diversity is so considerable, as in the case of the pus cocci, is largely dependent upon the multiplicity of its sources of entrance into the body.

The ability of the organism to produce a general febrile process which may terminate fatally has been well demonstrated by the clinical and bacteriological observations of the past few years. Ehlers§ was the first to draw attention to this by the publication of an instance of general systemic infection. His views received confirmation from Neumann¶ and Oettinger,|| both of whom reported cases of this nature. Later Karlinsky** published an interesting observation of generalized pyocyaneus infection in which the bacillus was obtained in pure culture from the skin vesicles, spleen, blood and Peyer's patches. Monnier†† studied an interesting example of this variety of infection in a case of broncho-pneumonia with pleurisy, in which he isolated the bacillus of blue pus from the heart's blood, pleural exudate and from the consolidated areas of broncho-pneumonia. Kossel,‡‡ Krannhals,§§ Calmette,¶¶ Williams,|||| Finkelstein,*** Barker††† and the author‡‡‡ have studied other illustrative cases of general pyocyanic infection in children and in adults.

Recently Triboulet§§§ observed an instructive case belonging to this

* *Semaine Méd.*, p. 385, 1890.
† *Compt. rend. Soc. de biol.*, p. 128, No. 4, 1896.
‡ Ibid., 1896.
§ *Hosp. Tid.*, Kjöbenh., 1890.
¶ *Arch. f. Kinderheilk.*, Hft. 1 and 2, 1890, xii, 1891.
|| Loc. cit.
** *Prager med. Woch.*, p. 231, No. 20, 1891.
†† See Legars. Essai sur la maladie pyocyanique chez l'homme. Thèse de Paris, 1895.
‡‡ Loc. cit.
§§ *Deutsche Zeitschr. f. Chirurg.*, xxxvii, 181, 1893.
¶¶ *Arch. de méd. navale et coloniale*, April, 1892.
|||| *Montreal Medical Journal*, July, 1894.
*** Loc. cit.
††† Loc. cit.
‡‡‡ *Philadelphia Med. Journal*, Sept. 17, 1898.
§§§ *Comptes rend. Soc. de biol.*, Oct. 22, 1897.

class in a child in which the systemic invasion was secondary to an impetigo and ulcerative ecthyma, from both of which lesions the pyocyaneus was isolated during life. The writer obtained from the laparotomy wound in a case of acute peritonitis the organism in pure culture and from the purulent peritoneal contents associated with a few colon bacilli. Cultures from the blood contained it in pure growth. From a case of acute pleuritis we also obtained it in pure culture. Jadkewitsch's case* is a most curious example of the nervous type of infection with this micro-organism.

Many of these instances of generalized invasion presented symptoms not unlike those observed in the experimental forms of infection; indeed, the similitude in some of the cases is most striking. A pronounced hæmorrhagic diathesis was noted in the majority of these cases, manifesting itself either in the form of hæmorrhages into the skin or sometimes into the internal organs, particularly into the intestinal wall. Diarrhœa was not uncommon and congestion of the intestinal mucosa of considerable intensity was noted in a few of the cases.

The infrequency of infection with this micro-organism is well shown by the fact that Krannhals† in the investigation of 30 cases of general septic and pyæmic infections was not able in a single instance to demonstrate the presence of the bacillus pyocyaneus. In the systematic bacteriological examination in 800 consecutive autopsies at the Johns Hopkins Hospital, Barker‡ found but 11 cases in which there was infection with this organism, sometimes manifesting itself as an acute local, at other times as a generalized process.

The objections advanced against the pathogenesis of the bacillus pyocyaneus in man by Schimmelbusch§ in his critical article certainly can no longer be regarded as tenable with the important affirmative evidence in our possession at the present day—all clearly demonstrating a more or less marked capability of the bacillus to produce lesions in the human subject.

The epidemic of dysentery, the report of which is the chief purpose of this paper, was investigated for the New York State Board of Health. For the clinical features of the cases we are indebted to Dr. M. C. Wright, of Mt. Vision, N. Y.

General Features.—The epidemic occurred at Hartwick, a

* Loc. cit.
† Loc. cit.
‡ Loc. cit.
§ Volkmann's *Samml. klin. Vorträge*, N. F., No. 62, 1893.

small hamlet in Otsego county, New York. It was limited to 15 individuals, with 11 recoveries and 4 deaths. 14 of these were members of two separate families living about one mile from each other. Little or no communication existed between the two houses. The first patient was attacked August 23, 1897, and the epidemic continued during the remainder of that month and the whole of September.

The disease first appeared in the S. family, composed of seven members, who all suffered from the affection, to which three succumbed, a male aged 35 and two children aged respectively 19 months and 5 years. The others made a good recovery after a long and tedious convalescence. The disease appeared in the second family about three weeks after the first member of the S. family was taken sick, and likewise attacked seven individuals, of whom six recovered and one, a child four years of age, died on the thirteenth day of the disease. The fifteenth case, it is interesting to note, was the physician in attendance on the S. family. On his first visit he drank freely of water, and three days subsequently was taken ill with symptoms in every manner identical with those presented by his patients.

Clinical Features.—Following is a short history of the clinical features of each case as given me by Dr. Wright:

Case 1. Duam S., age 35 years. Taken sick August 23 with pain in bowels and diarrhœal discharges which soon became bloody. Great tenderness over colon and marked tenesmus. Passages very frequent, one about every hour until death. Temperature ranged between 99° and 102° F. Death September 10.

Case 2. Milo S., age 19 months. Taken sick on same day as the first patient. Frequent bloody passages and great tenesmus. Much abdominal tenderness and considerable vomiting. Temperature 100°-103° F. Death August 26.

Case 3. A. S., 2 years. Illness commenced August 27. Symptoms milder than in either of the two preceding cases. Temperature did not go above 101° F. The duration of the disease was about three weeks and was followed by complete recovery.

Case 4. Ella S., age 37 years. Disease began September 1 and continued for about two weeks. Tenesmus and pain along line of the colon. Temperature at no time higher than 101° F. Recovery.

Case 5. Alice S., 5 years old. Taken sick September 7 and died on

the third day of her illness. Tenesmus marked and passages very bloody and frequent.

Case 6. Mrs. Duam S., 30 years. Taken sick September 10 with frequent passages (about 15 to 20 per day); tenesmus; temperature not over 100° F. Tenderness over large bowel was very acute, especially over cæcum. Vomiting quite frequent during first week. Recovery after 12 days' illness.

Case 7. Mrs. Van B., 50 years. Disease commenced September 14 and lasted about three weeks. Symptoms similar to those observed in Case 6, vomiting being perhaps still more severe. Practically no fever. Recovery after a very long convalescence.

Case 8. Mr. G. A., 20 years of age. Taken sick September 14 with diarrhœa, which quickly became bloody. Temperature on 15th reached 104° F., with a pulse rate of 120 to 130. For the first three days the condition was very critical, but after this improvement was slow but progressive, so that by the fourth week he was practically well.

Case 9. Mrs. W., about 28 years. In this case the symptoms were essentially those observed in the preceding cases, except that the clinical manifestations were less intense. Recovered in about two and one-half weeks.

Case 10. Mrs. W., 34 years. This patient had been nursing the S. family for a few days during their illness and shortly after returning home was in turn taken sick. The symptoms were severe for two days, but a marked amelioration followed shortly thereafter, so that by the end of the second week she had recovered.

Case 11. Florence C., aged 8. Sickness commenced September 17, and the symptoms continued for seven days. Attack very mild. Diarrhœa but no blood in stools. Recovery.

Case 12. Mr. C., 40 years of age. The sickness was ushered in with a chill. Diarrhœa for two days. Stools then became bloody. Marked tenesmus was present in this case. Frequent vomiting during first week. The disease lasted about six weeks, which was longer than in any of the other cases. Convalescence has been exceedingly slow.

Case 13. Maud C., 4 years. Illness began on the 24th of September and continued for twelve days. Frequent passages containing more or less blood, reaching often as many as 20 in a day. Vomiting and tenesmus very marked. Temperature not over 101° F. Hæmorrhage from the gums occurred in this case on the tenth day. Death on October 6th.

Case 14. Amos C., about 9 years old. Taken sick on September 26. Clinical picture practically same as in the other mild cases, except that patient passed more blood. Recovered.

Case 15. Dr. W., the physician to the S. family, was taken ill about September 1 with symptoms of a mild character, but of the same nature as those observed in the majority of the cases. Symptoms persisted for about nine days, when recovery took place.

It is seen, then, that all of the cases presented certain symptoms in common, so that the clinical pictures in the several instances bear a striking resemblance to each other. The onset was acute and the disease was ushered in sometimes with a chill, otherwise always with diarrhœal symptoms, which soon changed to those of a dysenteric type, characterized by frequent watery passages containing a greater or less amount of blood. Tenesmus and tenderness over colon were quite marked in some cases. Temperature was high in one instance (Case 8, 104° F.), but in the others it was never higher than 102° F. The duration of the disease in the different cases was quite variable, but the average was about three weeks for the non-fatal cases. One patient died on the third day of her illness. Vomiting, although not present in all cases, was, nevertheless, a marked symptom in many of the cases, particularly those presenting the more severe forms of the affection. An interesting and suggestive point which was noted about the physical appearance of the stools, but not mentioned in the histories, was the frequently distinct green color of the passages. This color became most evident after exposure to the air, and only rarely was it noticed at time of passage, and in these cases it was but faintly marked. Calmette in his studies had noticed this and explained it as due to oxidation of the pyocyanine contained in the intestinal dejecta. No autopsies could be obtained upon any of the fatal cases.

Hygienic Surroundings.—The two habitations are situated in each instance very favorably with relation to the formation of the surface of the soil, but the immediate surroundings and habits of each family are in every manner suggestive of an extremely low degree of hygienic morale, and close inspection and examination confirmed this in every way. In each family the well for the supply of drinking water is situated in close proximity to the kitchen door and is more or less imperfectly covered over with timber and earth. The soil over the well and immediately adjoining ground was covered by a layer of decomposing vegetable and animal detritus of offensive odor.

These substances were derived mostly from the kitchen, as it is the habit in each family to rid themselves of the refuse and other slops by throwing them, usually from the kitchen door, upon the ground in the vicinity of the supply of their drinking water. It is easy to understand that contamination of this well-water was a matter of no great difficulty, since the soil covering the well is only about two feet in depth and mostly made up of coarse sand through which penetration is not difficult. For none of the wells used for the supply of drinking water at the time of the epidemic could any other possible source of contamination be made out. At the C. house there exist two other wells located in a more favorable situation, but nevertheless readily liable to outside contamination by reason of their open character. Neither of these wells were used for the supply of drinking water at the time of the epidemic or previous to its occurrence.

The investigation was necessarily restricted in its extent owing to the distance of, and difficult accessibility to, the seat of the epidemic; for these and other reasons many points of importance and interest were unfortunately not determined.

Bacteriological Examination.—The microscopic and bacteriological investigation was limited to the examination of six specimens of intestinal discharges, four from the S. family and two from the C. cases. Three of these six specimens were from fatal cases, two from the S. cases and one from the fatal case in the C. family. The investigation also extended to the examination of five specimens of the drinking water, one specimen from each of the three wells at the C. habitation and two from the only well at the S. house.

A careful search for amœbæ was made in the specimens of intestinal discharges, with negative results in every instance. Amœbic dysentery is practically unknown in this section of the country.

Intestinal Discharges.—Cover-slip preparations stained by aniline gentian violet proved the predominating organism to be a bacillus of moderate length and thickness, showing little disposition toward any definite arrangement. A few other forms of bacteria were also present and resembled such micro-organisms as are usually observed in fæcal material. Epithelial cells, and in some cases some blood and granular debris, were likewise observed.

Gelatin and agar plates were made from all these specimens. Both sets showed an abundant growth of variously sized colonies which in the case of the gelatin plates always consisted almost exclusively of liquefiers. In three or four days the plates showed a greenish color throughout the medium. Further investigation demonstrated that the liquefying colonies consisted of a bacillus usually of the same morphological appearance when taken from different colonies in the same plate, but differing frequently in its morphology in different plates. Occasionally a discrete colony of a dirty white, brown or orange color was detected in the plates. A number of separate varieties of organisms was isolated: (1) a bacillus producing a green-colored pigment and in all the plates the most abundant organism; (2) another bacillus, a liquefier, but apparently producing no pigment, and (3) a non-liquefying and non-pigment-producing bacillus. Both of these latter varieties morphologically resembled, more or less, the predominating bacillus in the plates. A few saprophytic bacteria were also isolated.

The green pigment produced behaves in the following manner: In cover-slip preparations the organism proved to be a bacillus quite variable in its morphology when obtained from different media, and sometimes upon the same kind of media. The active motility of this organism was usually well observed in 20 to 30-hour bouillon cultures.

Agar-agar. Profuse, moist, greenish white growth along inoculation tract. After the second day the medium is diffusely colored with a more or less vivid green fluorescence.—*Potato.* Rust-brown, usually profuse growth. The chameleon phenomenon of P. Ernst was obtained.—*Bouillon.* Uniform turbidity with production of a slightly green film as culture becomes older.—*Dunham peptone solution.* Turbidity. Test for indol positive.—*Litmus-milk.* Acidification with precipitation of casein.—*Gelatin stab.* Liquefaction of gelatin with development of greenish color in gelatin.

Pyocyanine crystals were successfully isolated from various cultures by the method recommended by Charrin.

Animal experiments were made with this organism. 0.5 cc. of a 26-hour bouillon culture injected subcutaneously into a rabbit resulted in the production of a large inflammatory infiltration about the seat of

inoculation, with subsequent extensive sloughing of the superficial tissue. The animal survived. A similar dose was injected intravenously into a rabbit, and produced stupor and later death in about 30 hours. The autopsy showed congestion of lungs, kidneys, ecchymoses in liver and punctiform hæmorrhages in intestinal mucosa with congestion. The bacillus originally inoculated was isolated from the blood and viscera. The cultures used in the above inoculations were both derived from specimens of the intestinal dejecta. A guinea-pig received 1 cc. of a 20-hour bouillon culture (obtained from specimen of water) intraperitoneally. Stupor and slight diarrhœa and death on second day. Post-mortem examinations showed small hæmorrhages into peritoneum, pericardium and intestinal walls; congestion of latter as well as of kidneys; spleen swollen. The bacillus originally inoculated was cultivated from the abdominal viscera, but not from the blood.

Diagnosis.—Bacillus pyocyaneus (Gessard).

The extreme degree of variation noted in the morphology of the bacillus pyocyaneus has frequently been observed. This polymorphism was carefully studied by Charrin and Guignard* with particular reference to the conditions favoring its production.

The non-pigment-producing liquefier in cover-slips is seen to consist of a bacillus of variable size. This is also an organism of considerable motility.

Agar-agar. Moist, gray-white growth. Offensive odor.—*Gelatin stab.* Liquefaction usually well marked in 48 hours.—*Bouillon.* Uniform cloudiness of medium in 20 hours.—*Litmus-milk.* Rapid acidification and precipitation of casein.—*Glucose agar.* Abundant gas production.—*Saccharose agar.* Slight gas production.—*Potato.* Thick dirty-brown growth with strong trimethylamine odor.—*Dunham solution.* Turbid. Test for indol positive.

Diagnosis.—Proteus vulgaris (Hauser).

The third variety isolated was a short, thick bacillus, taking aniline-gentian violet stain well. Motility of the bacillus moderately active in 24-hour old cultures.

Agar-agar. Dirty white, sometimes brownish growth along tract of the needle.—*Bouillon.* Marked turbidity.—*Potato.* Abundant yellowish brown growth which later changes to darker color.—*Dunham solution.*

* *Académie des Sciences*, 12 Decembre, 1887.

Cloudiness. Indol reaction.—*Litmus-milk.* Acid reaction, precipitation of casein.—*Glucose agar.* Slight gas production.—*Saccharose agar.* Slight gas production.—*Gelatin stab.* No liquefaction.

Diagnosis.—Bacillus coli communis.

The bacillus pyocyaneus was present in almost pure culture in every specimen of the intestinal discharges examined, the bacillus coli communis in two specimens in very small numbers, and proteus vulgaris in three specimens, once in association with pyocyaneus and colon bacillus and twice with pyocyaneus alone. Associated with these three varieties was an exceedingly small number of colonies of common intestinal saprophytes.

The same methods of investigation were extended to the examination of the five samples of well water. The plates contained abundant numbers of liquefying colonies elaborating a green pigment, and further study demonstrated the colonies to be those of the bacillus pyocyaneus. In a specimen from one of the wells not used for drinking purposes by the C. family, a few colonies of the proteus vulgaris were isolated. All of these specimens in addition contained some common saprophytic water micro-organisms.

The probability of the causative relation of the bacillus pyocyaneus to the epidemic of dysentery is further strengthened by the fact that it is not a normal intestinal saprophyte. This has been well demonstrated by several investigators, and, indeed, its presence in the intestinal canal is not only very uncommon, but should be regarded with extreme suspicion, as was pointed out by Kossel.

Calmette, as a preliminary study to his investigations upon enterocolitis prevalent in Cochin-China, examined the stools of a large number of normal individuals in order to ascertain the exact nature of the normal microbic intestinal flora. In none of these bacteriological researches did he isolate the bacillus pyocyaneus, and therefore concluded that its presence in the intestinal tract cannot be regarded as normal. Kossel, from the results of numerous examinations of normal and pathological stools, confirmed the conclusions of Calmette. The presence of the organism in pathological conditions of the intestinal canal is by no means common. W. D. Booker,* in his work

* *Johns Hopkins Hospital Reports.* Vol. vi.

upon the summer diarrhœas of infants, found it only in occasional instances. Such an opinion receives additional confirmation from Krannhals,* who, in a bacteriological investigation of some 60 various intestinal disorders, only succeeded in isolating it once from an intestinal abscess.

The literature of dysentery referable to the bacillus pyocyaneus is extremely scanty. Outside of the researches of Calmette and Maggiora nothing can be found except isolated instances either of general or localized infection in which the intestinal symptoms were prominent, but generally associated with others of various kinds.

Calmette in the dysentery of Cochin-China found the bacillus pyocyaneus to be present in 15 cases out of 16 investigated, in the intestinal contents and ulcers in such abundance that it was practically the only bacterium which grew in the plates. From some of these cases it was also obtained from the blood in pure culture. The frequent association of the streptococcus with the bacillus pyocyaneus in the intestinal contents caused Calmette to attribute to the coccus the role of a predisposing factor in the production of the pyocyanic infection. Recently E. Phisalix† has published the results of experimental work which apparently demonstrates the increased susceptibility to pyocyaneus infection when associated with the staphylococcus pyogenes aureus. This is, however, true of many infections with other organisms.

Clinical evidence certainly shows that such infection is relatively more common as a secondary complication, and furthermore that age is a predominating factor. Young children in whom infection by pyogenic or other organisms is common, and where resistance is low, are those in whom pyocyaneus infection is most apt to occur. Infections with this micro-organism are not, however, confined to children, as many authors are inclined to believe, but are also relatively frequent in adults.

Patients suffering from the Cochin-China dysentery are affected with gastric disturbances, abdominal pains and frequent alvine evacuations, while not uncommonly a green discoloration of the intestinal

* Loc. cit.

† *Compt. rend. Soc. de biol.*, Feb. 27, 1897.

discharges is seen. The symptoms presented by the cases reported in this paper were in many respects not unlike those studied by Calmette.

In 1891 Maggiora,* in the dejections from 20 cases of epidemic dysentery which existed in the north of Italy, found colon bacilli and extremely virulent cultures of the bacillus pyocyaneus. Thiercelin,† and Williams and Cameron‡ found them in the pyretic and other forms of the gastro-enteritis of infants, as did also Lange.§

Of the small number of isolated cases of pyocyaneus infection which have been published from time to time, the following presented symptoms which were chiefly referable to the intestinal tract. Oettinger's¶ case was a young man who on the twentieth day of his convalescence from typhoid fever had what was apparently a relapse, but skin vesicles soon appeared and diarrhœa became very marked. Previous to the appearance of the vesicles constipation had existed. The pyocyaneus was obtained in pure culture from the vesicles and urine. Kossel observed in a four weeks old infant vomiting, diarrhœa and green stools. The patient died and cultures contained the bacillus in practically pure growth in the blood and intestinal discharges. Krannhals,‖ Finkelstein** and Triboulet†† have reported similar cases in which diarrhœal symptoms were prominent and the color of the stools was more or less markedly green.

The etiological relations of the bacillus pyocyaneus to epidemic dysentery is probably very infrequent, for Krannhals in the examination of 40 cases failed to find the bacillus in a single instance. A number of other investigators who have examined cases of dysentery have likewise failed to find it.

The bacteriological findings in the epidemic which occurred at Hartwick and which was studied by us, demonstrated the pres-

* *Centralb. f. Bact.*, 1891.
† Thèse de Paris. 1895.
‡ *Journal of Pathology and Bact.*, 1896.
§ See Heubner in Pentzold-Stintzing's Handbuch der Therap., vol. iv, p. 169.
¶ Loc. cit.
‖ Loc. cit.
** Loc. cit.
†† Loc. cit.

ence of the bacillus pyocyaneus constantly and in abundant numbers in every specimen of water and intestinal discharges. Furthermore, animal experiments showed this organism to be possessed of a considerable degree of virulence. Attention must also be called to the fact that the bacillus was not only the sole micro-organism present in great numbers in every case, but also that it was the only organism invariably found in the water.

The proteus vulgaris was isolated from two specimens of the intestinal discharges and once from a sample of water from a well not utilized for drinking purposes at the time of or previous to the outbreak of the epidemic. The bacillus coli communis was in no instance isolated from the water and only twice from the intestinal specimens.

The constancy of the presence of the bacillus pyocyaneus in both the discharges and drinking water, the numbers in which it was found and the virulence of the cultures furnish evidence of such a nature as to leave little doubt in the author's mind as to the etiological relation of the bacillus pyocyaneus in the production of this epidemic of dysentery. This view, it seems to me, is strengthened by th. ·milar observations of Calmette and Maggiora, and the not infrequent production of diarrhœas in children by this organism.

A HISTOLOGICAL STUDY OF TYPHOID FEVER.*

By F. B. MALLORY, M. D.

(From the Sears Pathological Laboratory of Harvard University and from the Pathological Laboratory of the Boston City Hospital.)

PLATES LIV-LXI.

The object of this paper is to throw some light on the primary, essential lesion of typhoid fever, and also on certain secondary lesions which result therefrom.

Little or nothing can be added to the classical descriptions by Hoffmann, Orth and others of the gross lesions occurring in typhoid fever. On the other hand, the descriptions of the histology of these lesions, of the cell changes in the various organs and of their relation to the general pathological process peculiar to typhoid fever, leave much to be desired.

The essential points which have been discovered in the histology of typhoid lesions can be summed up briefly.

In 1848 Virchow believed and taught that the swelling of the intestinal lymphoid tissue and of the mesenteric lymph nodes was due to a simple exudative process. Later, however, he convinced himself that the swelling was due entirely to hyperplasia of the cells of the lymphoid tissue, and vigorously opposed the view held by the Vienna school that it was caused by an albuminous exudation. He also considered that the swelling of the spleen was essentially hyperplastic in origin.

In 1857 Friedreich called attention to certain small circumscribed collections of cells in the liver and kidneys from a case of typhoid fever.

In 1860 and 1861 Wagner described more fully similar small grayish nodules which were found in the liver and kidneys, and subperitoneally over the ileum, cæcum and appendix in several cases of typhoid fever. Since his time the term "lymphoid nodules" has been applied to these collections of cells.

* Read before the Philadelphia Pathological Society, April 28, 1898.

In 1861 Billroth and Grohe, working independently, described certain very large multinucleated cells occurring in the mesenteric lymph nodes in typhoid fever. Both regarded them as examples of endogenous cell formation.

In 1862 Billroth called attention to the presence of these same large cells in the blood-vessels of the spleen and in the portal circulation. He traced the cells into the liver and concluded, after measurements both of the cells and of the liver capillaries, that it was possible for the cells to pass through the liver and reach the lungs. He suggested the possibility that the cellular foci of the liver described by Friedreich and Wagner might be due to emboli of these cells.

In 1869 Hoffmann observed these same large multinucleated cells in Peyer's patches and in the solitary follicles of the intestine in early cases of typhoid fever.

In 1875 Klein described them again in the intestine and mesenteric lymph nodes, with wonderful accuracy considering the technical disadvantages under which he labored, but he drew wrong conclusions from what he saw. He called attention to the large cells filled with red blood globules present in the spleen in typhoid fever.

In 1895 Reed claimed that the term "lymphoid nodules" applied to the focal lesions in the liver was incorrect, because these lesions were really areas of focal necrosis of liver cells invaded by polymorphonuclear leucocytes.

In recent years the histology of typhoid fever has been neglected for the typhoid bacillus and the serum reaction. Text-books on pathology pay little attention to the microscopic appearances of the early lesions of typhoid fever. The best, that of Orth, says that the essential cause of the swelling or new growth in the intestine is an accumulation in and around the lymph nodules of large cells, only in part of the character of ordinary lymph cells, and often containing many nuclei and occasionally red blood globules.

The latest views in regard to typhoid fever are those expressed by Ribbert in his Lehrbuch der pathologischen Histologie, published in 1896. He claims that the primary swelling in the intestine and mesenteric lymph nodes is due to the typhoid bacilli getting into the lymph sinuses and causing an acute inflammatory exudation of serum and leucocytes into the outer portion of the lymph nodules and the adjoining tissue. After a certain length of time the exudation disappears and is followed by an intense proliferation and desquamation of the endothelial cells lining the reticulum of the lymph spaces. These cells are occasionally phagocytic. The real lymphoid nodules of the lymphatic apparatus,

according to him, play no part in the process. Later, necrosis and sloughing of the newly-formed tissue occur, and repair by granulation tissue follows from the base of the ulcer.

A pathological lesion can be fully understood only when every step in its development, and especially its beginning, can be followed and studied. Where lesions can be produced experimentally this is a comparatively simple matter. For the lesions of typhoid fever, however, we are dependent on material obtained from post-mortem examinations, and we rarely have the fortunate combination of an early case of the disease and an early post-mortem examination, an absolute necessity for the perfect study of the lesions of the intestine. Other requisites are exact fixation of the nuclei, protoplasm and red blood globules, and a staining method which shall clearly differentiate the various tissue elements met with. For the fixation of the tissues Zenker's fluid has been found to give by far the best results, while for staining eosin followed by Unna's alkaline methylene-blue solution has afforded the clearest pictures.

During the past year I have been able to collect and to study histologically 19 cases of typhoid fever, 11 from the Boston City Hospital, 3 from the Massachusetts General Hospital through the kindness of Dr. J. H. Wright, and 5 from other sources. The duration of the disease in the cases examined varied from 10 days to 4 weeks.

The starting point of this investigation into the histogenesis of typhoid fever was a case of the disease in a girl eleven years old, who died at the Children's Hospital on the tenth day after the appearance of the first symptoms. The post-mortem examination was made one hour after death. The Peyer's patches and the solitary lymph nodules of the intestine showed marked swelling, and occasionally small yellowish foci due to necrosis, especially at the lower end of the ileum, but no macroscopic evidence of ulceration. The mesenteric lymph nodes were much enlarged and hyperæmic, and the spleen was considerably larger than normal, firm to the touch and dark in color. Portions of the intestine ware put at once, without washing the surface, into alcohol, Zenker's and Orth's fluids, and sections of the various organs were preserved in the same fixatives.

Although no other case was obtained so early in the disease or so

soon after death, yet it was possible in seven other instances to trace the whole development of the intestinal lesions, partly because the cell changes in typhoid fever are comparatively slow, and partly because all of the swollen Peyer's patches usually do not undergo necrosis and sloughing.

In the following histological descriptions of the various lesions in typhoid fever the different organs, beginning with the intestine, will be considered separately.

INTESTINE.

The lymph nodules of the intestine consist of masses of lymphoid cells lying in the meshes of a delicate connective-tissue reticulum. Lining this reticulum are certain large cells with vesicular, lightly staining nuclei, usually looked upon as the cells of the reticulum, but regarded by Ribbert and some others as endothelial cells intimately adherent to the reticulum, which is said to have a few spindle-shaped, deeply staining nuclei of its own. Owing to the close relationship between endothelial and connective-tissue cells embryologically and pathologically, and to the fact that the endothelium of newly-formed blood-vessels has been shown in the organization of thrombi to produce connective tissue, it is doubtful if these two varieties of cells can ever be sharply differentiated. In the lesions to be described, however, the large cells of the reticulum of the lymphoid tissue behave in all respects like the endothelial cells of the lymph and blood-vessels. This fact might be used as an argument, therefore, in favor of their being endothelial rather than connective-tissue cells. In the following pages these cells will be spoken of as endothelial cells.

Under the influence of the toxic product of the typhoid bacilli these larger endothelial cells increase in number (Plate LV, Fig. 4). The new cells are epithelial in character, with round or oval, vesicular nuclei surrounded by a considerable amount of protoplasm, which is always sharply outlined and is stained pink by the staining method employed. The proliferation of these cells may take place diffusely throughout the lymph nodule (Plate LIV, Fig. 2), but may also start in the centre (Plate LIV, Fig. 1) or at the periphery.

The cells increase not only in number but also in size, and soon

begin to incorporate (Plate LV, Fig. 5) and to digest the neighboring lymphoid cells. In a short time the only lymphoid cells left are those in that part of the lymph nodule where the endothelial cells have not proliferated. As these cells increase in size the nuclei lie eccentrically, usually near the periphery, and almost invariably become irregular in shape, either curved or variously indented. The cells are generally spherical in shape, but through pressure are often rendered irregular or elongated. Only rarely is a form seen that suggests amœboid movements on the part of these cells which probably do not change their relative positions, although they must have some power of motion in order to incorporate the cells found within them. Occasionally these large cells have two or more nuclei of their own.

These newly-formed cells all show to a marked degree the property of phagocytosis. Often 10 to 20 or more lymphoid cells in various stages of digestion will be found within one of the large cells (Plate LV, Fig. 6). As the lymphoid cells undergo digestion the nuclei shrink a little, become homogeneous in appearance and stain very deeply with nuclear stains. They usually dissolve irregularly and may become hollowed out into crescentic forms or break into fragments. As a rule, the incorporated cells can be seen to lie within digestive vacuoles. Occasionally red blood globules, more rarely plasma cells and polymorphonuclear leucocytes, are taken up by these large cells and digested in the same manner as the lymphoid cells. Outside of the lymphoid nodules, the less clearly defined lymphoid tissue shows an irregular, more or less diffuse formation of large cells with properties similar to those already described. The same cells are found in large numbers in the dense fibrous tissue of the submucosa, both beneath the patches of Peyer and the solitary lymph nodules, and also for some little distance peripherally beyond the limits of the lymphoid tissue. The cells are usually elongated, owing to pressure, and show marked phagocytic properties.

Orth has called attention to the swelling of the mucous membrane of the intestine, especially in the lower part of the ileum, in typhoid fever. Microscopic examination shows that the swelling is due largely

to the same cell changes that are found in the lymph nodules. They are most marked in the mucous membrane covering and adjoining the patches of Peyer, but also occur to a moderate degree everywhere in the mucous membrane of the lower part of the ileum. The changes are always found more marked between the glands of Lieberkühn (Plate LV, Fig. 3) than in the villi, and consist of proliferation of the endothelial cells in the fibrillar reticular connective tissue, the meshes of which in this situation contain usually more plasma than lymphoid cells and also a fairly large number of eosinophiles. The newly-formed cells do not differ in character from those already described.

Proliferation of the endothelial cells is shown by mitosis in all of the situations in which these cells are found (Plate LV, Figs. 3 and 4). While not abundant, it is sufficiently frequent to account for all of the new cells formed.

Besides these cells derived by proliferation from endothelial cells and characterized by marked phagocytic properties, there are certain other cell changes which have to be taken into consideration. The epithelial cells in the glands of Lieberkühn are in a state of very active proliferation, as shown by numerous mitotic figures present in every gland. How much more abundant, if at all, the cell division is than in the normal intestine it is impossible to say. Occasionally a gland of Lieberkühn is dilated and filled with polymorphonuclear leucocytes. Mitosis is also very frequent in the lymphoid cells remaining in the lymphoid tissue and in the mucous membrane. The mitotic figures can be differentiated from those of the endothelial cells because they are smaller and by the fact that their protoplasm is very granular and stains deeply with methylene-blue. Many of the lymphoid cells have changed or are changing into plasma cells characterized by small, round, coarsely granular, deeply staining, eccentrically situated nuclei and finely granular protoplasm which stains deeply with methylene-blue. Besides the lymphoid and plasma cells originally in the tissues or derived from them, others are added to them by emigration from the blood-vessels (Plate LVI, Fig. 14), Large multinucleated plasma cells are found occasionally. Irregu-

larity of shape, due to amœboid movements, is shown by many of the lymphoid and plasma cells.

In the muscular coats of the intestine the cellular increase is most noticeable along the lymphatic vessels. The cells consist in part of infiltrating lymphoid and plasma cells, but principally of large phagocytic cells in the lymph vessels and spaces around and near the blood-vessels. Similar cells are also found between the smooth muscle fibres and are generally elongated in shape in consequence of pressure. Mitotic figures can occasionally be found, and the cells often occur in small clumps.

Subperitoneal collections of cells are usually found only beneath the patches of Peyer. As in the muscular coats, they consist of collections of phagocytic cells derived by proliferation from the endothelial cells around the blood-vessels. Occasionally fairly large clumps of cells are formed.

LYMPHATICS.

The terminal lymphatics of the mucous membrane of the intestine by the tenth day of the disease are distended and filled with large phagocytic cells derived from the lining endothelium (Plates LV, Fig. 3, and LVI, Fig. 8). Along with them is usually a small number of lymphoid and plasma cells. In consequence of their distention with cells, it is easy to follow the lymphatic vessels into and all through the submucosa (Plate LV, Fig. 7). The cellular contents vary to some extent in the relative number of phagocytic and lymphoid cells, but as a rule the large cells predominate.

BLOOD-VESSELS.

Microscopic examination of the distended blood-vessels of the mucous membrane and submucosa shows a surprising absence of polymorphonuclear leucocytes. In their stead are occasional lymphoid and plasma cells, which not infrequently divide by mitosis in the blood current, and very numerous, large cells with lightly staining, vesicular, curved or indented nuclei (Plate LVI, Fig. 10). Many of these cells are phagocytic and contain red blood globules, less often lymphoid and plasma cells or polymorphonuclear leucocytes.

The number of cell inclusions is usually small and is limited chiefly to red blood globules. The large cells are evidently derived from the vessel walls, because examination of the lining endothelium of the capillaries and veins of the mucosa and submucosa shows mitotic figures to be quite numerous (Plate LVI, Figs. 9 and 12). During mitosis the cells are usually adherent to the wall but may be somewhat loosened. Much more rarely these large cells divide by mitosis when free in the blood stream.

The lesions thus far described are proliferative in character and probably mark the limit reached by the typhoid process in many of the agminated and solitary lymph nodules in mild cases of the disease. Even in severer cases some of the patches of Peyer do not undergo necrosis with sloughing and ulcer formation. Instead, the large newly-formed phagocytic cells rapidly undergo fatty degeneration and disappear. When the typhoid process has been more severe, however, certain changes take place which lead to obstruction of the lymphatics and to the obliteration of many of the blood-vessels, thereby causing necrosis of the tissue in which they lie.

Virchow thinks that the necrosis is due to a form of caseation much like that occurring in tuberculosis, while Orth is of the opinion that the necrosis is due to pressure exerted on the blood-vessels in consequence of the great hyperplasia of the cells. Careful study leads me to the view that possibly some of the capillaries may become occluded by proliferation of their endothelial cells, but the truth of this view or of Orth's is difficult to demonstrate. By far the most common method of occlusion of the blood-vessels, and perhaps the only one, takes place in the following way:

The first regressive changes in the intestinal lesions of typhoid fever are shown in the large phagocytic cells in the lymphatic vessels. The cells degenerate and an abundance of fibrin soon binds them together. The outlines of the cells disappear and the nuclei become more irregular than before. In this way thrombi are formed which occlude the vessels, so that it is difficult to believe that much, if any, circulation can take place in them under such circumstances.

Beneath the lining endothelium of the large and smaller veins,

much more rarely of the larger lymphatic vessels, accumulations of cells take place in what must be a lymph vessel or lymph space. The cells consist in part of lymphoid and plasma cells, in part of large phagocytic cells derived by proliferation from the cells lining this lymph space (Plate LVII, Fig. 15). The cell accumulations, which project more or less into the lumina of the veins, may be large or small, focal in character or spreading out for some distance around or along the vessels. Necrosis of the large phagocytic cells takes place very frequently in these collections of cells beneath the endothelium and furnishes the starting point for a very abundant formation of fibrin which often extends for some distance along the vessel (Plate LVII, Fig. 16). The nuclei of the large cells undergoing necrosis usually become more irregular in shape than normally before they lose the property of staining and disappear. The lining endothelium of the vessel is generally preserved intact (Plate LVII, Fig. 19), at least for a time, over such a collection of cells, but often by necrosis becomes the starting point for the formation of fibrin which reaches out into the lumen and by extending along it may divide it into two or more channels. The cells in the blood current get caught in this fibrin and thrombi are soon formed which completely occlude the vessel (Plate LVII, Fig. 17). From these primary foci the fibrin formation extends along the vessels and into the capillaries, thereby occluding them. So far as can be ascertained by a careful study of serial sections, these masses of fibrin, which are often very numerous beneath the lining endothelium of veins before any other regressive changes have taken place anywhere in a Peyer's patch, always start from the protoplasm of degenerating phagocytic cells. The fact that these large cells do form beneath the lining endothelium of the veins is best seen in the larger vessels of the spleen (Plate LXI, Fig. 34) and liver. The areas thus deprived of their blood supply by occlusion of the veins and capillaries soon show evidence of undergoing necrosis. The outlines of the cells become indistinct and more or less fused, owing to the formation of fibrin which is not always very abundant. The nuclei become even more irregular in shape than normally and finally disappear. The included, partially digested cells break up into fine particles which after a while refuse to stain.

The inflammatory exudation which takes place in consequence of the necrosis of the tissue consists almost wholly of serum in which abundant fibrin forms. Polymorphonuclear leucocytes are rarely found. The exudation, as is usual in the intestine, collects in greatest quantity in the deeper layers of the submucosa. The earliest necroses seem to take place generally in the lymph nodules which were first affected. The mucous membrane also, which is very thin over them, quickly undergoes necrosis. The upper portions of these necroses soon macerate and slough off. In this way small superficial ulcerations are formed, along the edges of which numerous bacteria quickly collect. Sometimes, however, the mucous membrane over a Peyer's patch is fairly well preserved when necrosis in the submucosa is already well advanced. In the small necroses polymorphonuclear leucocytes are very uncommon, but in the large necroses they usually occur in considerable numbers, not along the edge of the necrotic tissue, but nearer the surface where the various bacteria of the intestinal tract are invading the necrotic tissue. The irregularity of the necrosis and ulceration of a Peyer's patch depends, of course, on the fact that all of the veins usually do not become occluded. Even when the necrosis is quite marked there is generally a little tissue immediately around the arteries supplying the affected areas which does not undergo necrosis, at least at first, because the arteries, unlike the veins, do not become thrombosed.

MESENTERIC LYMPH NODES.

The lesions occurring in the mesenteric lymph nodes do not differ in character from those found in the lymph nodules of the intestine. In fact the mesenteric lymph nodes offer the very best tissue for studying the essential lesion of the typhoid process in all its stages, because they are not so freely exposed to invasion by various organisms as is the intestine the moment its lining epithelium is injured.

In the lymph nodes the lymph sinuses are always more affected than the nodules and cords of lymphoid cells. They become widely dilated and filled with large phagocytic cells containing mainly lymphoid and plasma cells, but also occasionally red blood globules. Besides

the large cells, numerous lymphoid and plasma cells are also present in the sinuses. The large cells are unquestionably derived in part, if not entirely, from the endothelial cells lining the loose reticulum of the sinuses.

In the lymphoid tissue between the sinuses a proliferation of the large cells lining the reticulum takes place just as in the intestine. Sometimes it is most marked centrally, sometimes diffusely or peripherally. Mitosis of the endothelial cells of the reticulum of the sinuses and lymphatic tissue is fairly frequent. Necrosis in the lymph nodes, when it occurs, is always most marked and most extensive in the lymph sinuses. An abundant formation of fibrin takes place, binding the cells together. The nuclei of the large cells become very irregular in shape and finally disappear.

In the lymph nodules the necrosis seems to depend largely on the same lesions of the blood-vessels that are found in the intestine, namely, collections of phagocytic and lymphoid cells beneath the lining endothelium, necrosis of the large cells and fibrin formation with extension into the vessel and occlusion of the lumen.

The typhoid process often extends outside of the lymph nodes into the adjoining fat tissue, where many large phagocytic and plasma cells are found between the fat cells. These areas also may become necrotic.

In many cases some at least of the lymph nodes do not undergo necrosis; instead, resolution takes place; the cells rapidly show signs of fatty degeneration and disappear. Apparently resolution can occur even when fairly extensive necrosis with fibrin formation exists in the lymph sinuses. What the exact changes are which take place when a whole lymph node becomes necrotic I have not been able to follow. Certain it is, however, that the necrotic tissue ordinarily shows very little or no infiltration with polymorphonuclear leucocytes.

SPLEEN.

I wish to speak next of the spleen, because the cell changes which take place in it have an important bearing on the lesions of the liver.

As a rule the lymph nodules of the spleen do not show any marked change in typhoid fever. In certain cases, however, some or all of

the nodules show to a greater or less extent the same formation of phagocytic cells from the endothelial cells of the reticulum that is found in the lymphoid tissue of the intestine.

The most marked changes of the spleen are those found in the blood-vessels and spaces of the pulp. They are much dilated and filled with large phagocytic cells containing principally red blood globules, usually in very large numbers, but also lymphoid and plasma cells and polymorphonuclear leucocytes. These phagocytic cells are derived chiefly or entirely by proliferation and desquamation from the endothelial cells lining the vessels. Occasionally mitosis takes place in the cells lying free in the blood. Sometimes the blood-vessels and spaces become occluded by masses of these large cells, which degenerate and become bound together by a meshwork of fibrin.

Besides this general proliferative process we get, as in the intestine, numerous plasma cells in the veins and in the splenic pulp. Many of them show amœboid shapes and not infrequently mitosis.

In some of the splenic veins, especially the larger ones, the lining endothelium is lifted up by masses of large phagocytic cells, and of lymphoid and plasma cells (Plate LXI, Fig. 34). In places these cells degenerate and fibrin forms between and around them.

LIVER.

Two varieties of focal lesions, to both of which probably the term "lymphoid nodule" was applied originally, are often found in the liver in typhoid fever. One is confined to the small amount of connective tissue around the portal vessels, that is, it lies outside of the lobules and in no immediate relation to the liver cells; the other may occur anywhere within the lobule among the liver cells. The two forms of lesion often adjoin each other. It is important to separate them sharply because they arise in different ways.

The lesion around the portal vessels consists to some extent of an increase of lymphoid and plasma cells, due in part to proliferation, in part, probably, to immigration. The chief part of the lesion, however, is formed of large cells with phagocytic properties derived by proliferation from the endothelial cells around the portal vessels. The

phagocytic cells do not differ from those found in the intestinal lesions and in the mesenteric lymph nodes, except that the cell inclusions are not so numerous and are confined almost entirely to lymphoid cells. Very rarely a giant cell is formed in this situation, apparently by fusion of the endothelial and other cells in a lymph vessel.

In order to understand the origin of the second form of focal lesion in the liver—that occurring somewhere within the lobule among the liver cells—it is necessary to refer to the lesions of the intestine and spleen. In these organs we found that fairly abundant mitosis of the endothelial cells of the capillaries and veins took place, that these cells desquamated into the blood current, and in the intestine could be followed into the large veins of the submucosa. They were distinguished from the nucleated cells ordinarily found in the blood by their size, the irregular shape and eccentric position of their nuclei, and especially by their marked phagocytic properties. The last feature was most prominent in the cells in the veins and blood spaces of the spleen, where the circulation is probably slower. From these organs many of the large cells are carried directly to the liver. Billroth observed large multinucleated cells in the splenic and portal veins in 1861 and traced them to the liver. As already mentioned, he thought they might be the source of the lymphoid nodules in the liver described by Wagner. He does not seem to have recognized the fact that the large cells often contained red blood globules.

Examination of the livers of the cases that came to post-mortem examination within the first two weeks of the disease shows in many of the branches of the portal vein, numerous large phagocytic cells containing usually many red blood globules or undigested portions of them, less frequently lymphoid cells, and rarely polymorphonuclear leucocytes (Plate LVIII, Fig. 21). These same cells are found in the capillaries all through the liver, often closely crowded together. Mitosis may take place in them either in the large vessels or in the capillaries (Plate LVIII, Fig. 23). The cells often assume irregular and elongated shapes, evidently from the pressure to which they are subjected in passing through the narrow vessels. Occasionally these cells undergo necrosis, as is shown by the fact that the

various stages in the cell changes leading up to the picture presented by complete necrosis can be followed, and in these earlier stages the cells can be definitely recognized by the inclusions in the protoplasm.

Occasionally some of these large cells stick fast in the capillaries. At least four causes favor this condition, viz.: the general swelling of the liver cells from parenchymatous degeneration leading to narrowing of the capillaries, the very large size of many of these phagocytic cells in consequence of the great number of their inclusions, the swelling and phagocytosis of the endothelial cells lining the liver capillaries, and finally, mitosis of the capillary endothelium (Plate LVIII, Fig. 20).

When by obstruction of a capillary a focus of cells is formed, other cells are added to it, partly, it would seem, from mechanical causes owing to interference with the circulation at such a point, partly from cell division as is shown by mitosis, so that soon a number of liver cells are completely surrounded by these large cells (Plate LVIII, Figs. 22 and 25). Then the included liver cells, presumably on account of the interference with their nutrition, quickly undergo necrosis, as is shown by the deeper stain of their protoplasm with eosin (Plate LIX, Fig. 27). The nuclei at first stain deeply (Plate LIX, Fig. 27), then they and the protoplasm break down into granular detritus and disappear (Plate LX, Fig. 29). Sometimes the cells which block up the capillaries undergo necrosis before the liver cells (Plate LX, Fig. 28).

Pressure on the surrounding liver cells, making them narrow and elongated, is often exerted peripherally by these clumps of cells owing to the proliferation in them. It is possible that some of the foci in the liver may arise solely from the proliferation of the endothelial cells lining a capillary. The cells within the focal areas usually do not contain many inclusions, probably because those which they may have taken up in the circulating blood have been more or less digested, and because the newly-formed cells in the foci do not have the same opportunity for getting hold of other cells. Mingled with these large cells in the focal areas are often a few lymphoid and plasma cells, and occasionally red blood globules.

Sooner or later necrosis of these large cells in the focal areas takes place, possibly not always, for it is presumable that at least the smaller collections of cells may undergo fatty degeneration and disappear as in the intestine and mesenteric lymph nodes. The necrosis begins in the centres of the areas; the protoplasm loses its sharpness of outline, fibrin usually appears between the cells and binds them together (Plate LIX, Fig. 26), the nuclei of the large cells become still more irregular in shape and finally disappear. Invasion of the necrotic areas in the liver with polymorphonuclear leucocytes even as late as the fourth week is infrequent and slight. What becomes of the areas after this time could not be ascertained owing to the lack of appropriate material in which to trace the process.

This form of focal lesion, accidental in its location in a liver lobule and leading to the destruction of a limited number of liver cells, is not confined to typhoid fever, but occurs in other infectious processes. It may also result from cell emboli derived from malignant growths. The same variety of lesion can be produced experimentally in 24 to 48 hours by injecting a sterile suspension of carmine in normal salt solution into a mesenteric vein of a guinea-pig.

There is a form of primary necrosis of liver cells which occurs in many acute infectious processes, and is due apparently to some strong, diffusible toxine. It follows practically the same distribution as that seen in chronic passive congestion, *i. e.* the necroses are confined fairly definitely to the centre of every lobule. The capillaries remain pervious, the necrotic liver cells are quickly invaded by polymorphonuclear leucocytes. It was produced experimentally by injecting a dose of diphtheria toxine sufficiently large to kill a rabbit in three days. The muscles all over the body showed intense fatty degeneration and the centre of every lobule of the liver was necrotic. No example of this type of lesion was found in any of the cases of typhoid fever.

Many of the phagocytic cells pass through the capillaries of the liver and get into the lungs, where they can be found in the larger and smaller blood-vessels and in the capillaries (Plate LXI, Fig. 33). They do not seem to cause any obstruction, but pass along into

the general arterial circulation. Sections of the pale clots of blood from the right ventricle of the heart in a case that died on the fourteenth day of the disease showed numerous large phagocytic cells among the normal cells of the blood. Sections of a clot from the left auricle showed them present there also in about equal numbers (Plate LXI, Fig. 32). There is no question, therefore, about these cells getting into the general arterial circulation. Examination of the pale watery fluid in the thoracic duct in this same case showed an occasional large phagocytic cell containing numerous lymphoid cells. It is evident, therefore, that cells also get into the general circulation through the thoracic duct (Plate LXI, Fig. 30). The cells derived from the lymph vessels show almost nothing but lymphoid cells as inclusions, while those that come from the portal circulation contain chiefly red blood globules.

It is difficult to explain why these large cells have not been found in the ordinary blood examinations during the second and third weeks of typhoid fever, when they are most abundant, except on the ground that the dried cover-slip method of examining blood breaks up cells of this type. In 1874 Eichhorst reported finding from 2 to 4 large cells each containing 2 to 5, rarely as many as 7, red blood globules in every drop of blood drawn from the tip of the finger of a typhoid patient. This condition was present for five days during the second week of the disease, after which time the large cells suddenly disappeared. Slight pressure upon them caused these cells to break up and set the globules free. I have found them on careful examination in the blood-vessels of the heart, kidneys and brain. So far as can be judged from the sections of the clots in the heart, the phagocytic cells may form at least from 1 to 2 per cent of the nucleated cells in the circulation.

The kidney is the only organ in which the phagocytic cells in the general circulation seem to give rise to any lesion.

It has been shown by Dr. W. T. Councilman* that when the lymphoid and plasma cells increase in number in the general circulation, they filter out into the veins in the pyramids of the kidneys, and

* *Journal of Experimental Medicine*, iii (1898), 393.

may often be there in great numbers. Under certain conditions these cells emigrate from the vessels and give rise to focal collections of cells. In typhoid fever the large phagocytic cells filter out in the same way and crowd the vessels along with numerous lymphoid and plasma cells. Occasionally the large cells occlude several adjoining blood-vessels, and by cutting off the circulation lead to necrosis of the intervening tubules. To complicate the picture, emigration of the lymphoid and plasma cells also present may take place, and hæmorrhage is not infrequent.

The heart was examined in two cases, but in only one was the examination at all thorough. In both cases occasional small foci of lymphoid and plasma cells were found similar in character to the areas often seen in the heart in diphtheria. Besides this form of lesion, phagocytic cells were found here and there in the lymph spaces, especially beneath the pericardium and along the larger blood-vessels. In some of the lymph vessels they were quite numerous. Occasionally a lymph vessel would be occluded by them and then the adjoining lymph spaces would usually also be crowded with them. In this way foci of considerable size would be formed. In one place the lining endothelium of the endocardium was lifted up by a group of large cells (Plate LVII, Fig. 18). In none of these areas was necrosis found. In one brain a single focal lesion was found, but the tissue had been hardened in alcohol and the character of the cells could not be determined. In two cases an occasional phagocytic cell was found in the lymph spaces of the pia-arachnoid.

Examination of the testicles in two cases showed complete cessation of spermatogenesis. One case showed nothing else. In the other the blood-vessels were dilated. Everywhere in the connective tissue between the tubules were numerous large cells in the lymph vessels and spaces. The cells were large and had irregular nuclei and abundant protoplasm; they were often phagocytic and occasionally showed mitotic figures. Sometimes the large cells were clumped together, apparently in connection with the large lymph vessels. In this same case there were numerous fairly large foci of lymphoid cells with a certain proportion of large phagocytic cells among them. Plasma

cells and polymorphonuclear leucocytes were rare. Scattered diffusely in the tissue were numerous Mastzellen.

The bone-marrow from the femur was examined in two adult cases, in both of which it was found quite red at the post-mortem examination. The cell changes closely resembled those in the spleen. In the blood-vessels were many large cells containing red blood globules, much less frequently lymphoid cells and polymorphonuclear leucocytes. The large cells also occurred diffusely in the lymph spaces among the other cells of the bone-marrow and around the fat cells. Occasionally clumps of these phagocytic cells were found undergoing necrosis and bound together by fibrin. So far as could be determined, the areas arise by occlusion of blood and lymph vessels by these large cells.

The bladder examined in one case was negative, while the wall of the gall-bladder was infiltrated with numerous lymphoid and plasma cells, and occasionally showed phagocytic cells.

Summing up the lesions thus far described, we have as the essential lesion of typhoid fever a diffuse proliferation of endothelial cells giving rise to large epithelioid cells characterized by the possession of marked phagocytic properties. This proliferation is most marked in the lymphoid tissue of the intestine, in the mesenteric lymph nodes, in the spleen, bone-marrow and liver, but may also be found to a varying extent in the lymphatic vessels all over the body, as shown by examination of the lymphatics of the heart, lungs, testicles and pia-arachnoid.

In regard to the typhoid bacillus I have very little to say and nothing new to add. It was obtained in cultures from most of the organs of the cases studied. Histologically it was rarely found, but it must be confessed that no exhaustive search for the organism was made. Occasionally the characteristic colonies were found in the mesenteric lymph nodes and in the spleen, but not in any case that came to post-mortem examination very soon after death. In the earliest case, in which the post-mortem examination was made one hour after death, no bacteria were found microscopically except along the edges of the beginning ulcerations of the intestine, and in that

situation it is of course impossible to differentiate the typhoid from the colon bacillus. Certain it is that when the typical colonies of the typhoid bacillus are found in the different organs they bear no intimate relation to the lesions present.

It would be exceedingly interesting to know whether the typhoid bacillus gains access to the lymphoid tissue of the intestine before any lesion is produced and before the lining epithelium is injured, or whether the lesion is caused primarily by absorption of a toxic substance eliminated by the bacilli which at first are within the intestinal canal only and which gain an entrance into the tissues later.

Unfortunately the line of experimental work so successfully carried out with the diphtheria bacillus and its toxine seems to be out of the question with the typhoid bacillus owing to the immunity in respect to this organism enjoyed by the lower animals. We have to depend, therefore, as yet, for our views in regard to the typhoid bacillus and the lesions produced by it on such deductions as may be drawn from the study of the gross and histological lesions occurring in man.

The following facts favor the view that the intestinal lesions are due primarily to an absorption of a toxic substance secreted by typhoid bacilli in the lumen of the intestine. The lesions diminish in intensity from the ileo-cæcal valve upwards, and are along the line of absorption. The lesions are diffuse. This characteristic is shown in three ways: (*a*) all of the lymphoid tissue for a given distance is generally, although not equally, affected; we do not get here and there a patch of Peyer swollen while the intervening patches remain normal; (*b*) the whole of each patch of Peyer affected shows about the same degree of swelling; (*c*) the mucous membrane in the lower part of the ileum shows diffuse proliferation. In judging of the above points it is necessary to take early cases in which the lesions are in the stage of swelling, because the ulcers, as already shown, depend on more or less accidental causes, which may lead to ulceration of half of a patch of Peyer while the other half undergoes resolution, or to complete ulceration of one or more patches while the others return to normal conditions.

This view that the intestinal lesions are due primarily and perhaps

chiefly or entirely to a toxine originating in the intestinal lumen is favored by the character of the lesions occurring elsewhere in the body. They all lie along the lines of absorption through the lymphatics and of distribution through the circulation; they vary in intensity and are always diffuse; an organ, such as the spleen or a mesenteric lymph node, shows practically the same degree of cell change throughout its whole extent. The focal lesions in the liver and other organs all depend on accidental causes.

If the lesions depend on the immediate presence of the typhoid bacillus, then we have to assume that multiple infections of the lymphoid tissue of the intestines take place within a very short time, extend from the ileo-cæcal valve upward and spread uniformly throughout each patch of tissue affected; also that the organism gets into the circulation and reaches the spleen and other organs in the very beginning of the process.

Ribbert bases his opinion that the typhoid process begins with an acute inflammatory exudation on the fact that he finds among the large cells in early cases a little fibrin and an occasional leucocyte which he regards as the remains of the exudation. In view of the lesions above described, it seems more reasonable to regard this evidence of inflammation as the beginning rather than the end of the inflammatory process which is secondary to degeneration of the proliferated cells. Neither can his view that the newly-formed cells are of a reparative nature be accepted; for these cells do not produce any intercellular substance, or form blood-vessels or connective tissue as endothelial cells do in the organization of thrombi and in other reparative processes; instead they either undergo fatty degeneration as in resolution, or extensive necrosis (for which he gives no explanation), followed by ordinary granulation tissue.

The multinucleated lymphoid cells of the early observers are for the most part the phagocytic cells with their inclusions, produced by the action of the typhoid toxine. A very few of them, however, are multinucleated plasma cells.

The typhoid process in the various organs, especially the intestine, spleen and bone-marrow, is to a slight degree complicated by the form-

ation of plasma cells from lymphoid cells, by proliferation of lymphoid and plasma cells, and by their migration into and out of blood-vessels and in the tissues. These lymphoid elements never reach anything like the number present in cases of diphtheria and rarely give rise by emigration to the focal and diffuse collections of cells so often found in that disease, particularly in the kidneys. It is a question whether their increase is due to the direct action of toxic substances or is to be regarded as reparative in nature and dependent on other causes.

This formation by proliferation of phagocytic cells from endothelial cells is not peculiar to typhoid fever except in regard to location, extent and degree. The fact that toxic substances derived from various sources may produce at least two effects on cells, the one degenerative, the other proliferative, and also that they may act focally or diffusely, seems generally to have been overlooked. The staphylococcus pyogenes aureus is a good example of an organism that produces necrosis focally. The toxine is strong and is but slightly diffusible. The tubercle bacillus on the other hand produces proliferation focally. The diphtheria bacillus acts both focally and diffusely and produces, according to the strength of its toxine, either proliferation or necrosis, or the one process following the other. The typhoid bacillus produces a mild toxine that acts diffusely and causes proliferation.

Taking this view of the action of the typhoid bacillus, that it causes proliferation of endothelial cells by means of a diffusible toxine, how are the cases of meningitis, of pneumonia and of abscess formation in bones, the spleen and other organs, reported to be due to the typhoid bacillus, to be explained?

In the spleen of one of the cases studied there were two yellowish nodules, one the size of a pea, the other of a marble. On section the larger nodule was opaque and yellowish white in one-half, while the other half contained thick pus. The smaller nodule was firm except near the centre, where a little pus was present. Bacteriological examination showed only the typhoid bacillus present both in the abscesses and elsewhere in the spleen. On microscopic examina-

tion numerous small areas of large phagocytic cells undergoing necrosis and imbedded in abundant fibrin were found all through the spleen. In the neighborhood of the nodules just mentioned some of the large veins were thrombosed by masses of fibrin and degenerating phagocytic cells. The firm portions of the nodules consisted of necrotic tissue in which in places degenerating phagocytic cells could still be distinguished. The softened portions of the nodules contained polymorphonuclear leucocytes. Typhoid bacilli were present in moderate numbers in the form of colonies in the necrotic tissue, and scattered diffusely in the softened portions. In the latter situation many of them were within the leucocytes. This case suggests that possibly the typhoid bacilli, which happened to be in the necrotic area produced through occlusion of blood-vessels by phagocytic cells, may have found more favorable surroundings for growth than they ordinarily do in living tissue, just as the tubercle bacillus grows most luxuriantly in old cavities of the lung or in softened, broken down lymph nodes. It is also possible that the post-typhoid abscesses of bones may owe their origin through obstructed blood-vessels to primary foci of necrosis, in which, as in the necroses in the spleen, the typhoid bacillus finds favorable surroundings for growth.

One of the cases of typhoid fever was complicated with typical fibrinous pneumonia of the whole of the right lower lobe in the stage of gray hepatization. The post-mortem examination was made in a hurry, at night, three hours after death, and unfortunately cultures of the lung were not made. Microscopic examination of the exudation showed serum, fibrin, fairly numerous polymorphonuclear leucocytes, occasionally lymphoid and plasma cells, rarely an eosinophile. There were also present, however, great numbers of large phagocytic cells with irregular, vesicular nuclei situated peripherally (Plate LXI, Fig. 31). The inclusions consisted almost entirely of polymorphonuclear leucocytes, of which usually one or two, but occasionally several, were within a single cell. More rarely the inclusions consisted of red blood globules. The number of phagocytic cells varied considerably in different parts of the lobe, but in many of the alveoli this was almost the only cell present. Microscopically, pneu-

mococci were present in very small numbers, while a bacillus, morphologically like the typhoid bacillus, occurred more abundantly and usually within leucocytes. The perivascular and peribronchial lymph vessels and spaces contained lymphoid, plasma and phagocytic cells in small numbers. There are of course three possible sources for these phagocytic cells within the alveoli of the lung. They may have emigrated from the blood-vessels or have been derived by proliferation from the epithelium lining the alveoli or from underlying endothelial cells after destruction of the epithelium. The third view seems the preferable one. No evidence of emigration or of active amœboid motion on the part of these cells has ever been observed. On the other hand very numerous mitotic figures were found in large cells lining the alveoli. It is of course impossible to deny that the proliferating cells may be epithelial, but in view of the marked phagocytic properties shown by them, it seems more reasonable to assume that they have the same origin as the other phagocytic cells already described. This is assumption, however, not proof.

The simplest explanation of the presence of these phagocytic cells in such large numbers (for they can be found not infrequently in ordinary cases of pneumonia) is that we have here a case of lobar pneumonia complicated by the action of typhoid bacillus of which the toxine has caused a proliferation of certain cells in the wall of the alveoli. How the typhoid bacilli get into the lung I do not pretend to explain beyond suggesting that they may have been brought from the blood-vessels by the emigrating leucocytes. These two cases, the abscesses in the spleen and the lobar pneumonia, certainly suggest that careful bacteriological and histological study of the various complications of typhoid fever, and especially of cases of meningitis claimed to be due to the typhoid bacillus alone, may throw additional light on the character of the lesions produced by the typhoid bacillus.

In view of the lesions described in the blood-vessels of the intestine, liver and spleen, and beneath the lining endothelium of the heart, it seems fair to suppose that the thrombi which sometimes occur in typhoid fever, rarely in the heart but rather frequently in the veins of the lower extremities, may owe their origin to similar lesions.

Remlinger has recently claimed to have produced typical typhoid lesions in a certain proportion of rabbits and guinea-pigs by feeding them every day on vegetables soaked in bouillon cultures of typhoid bacilli. A series of experiments was carried on to confirm, if possible, these results, but without success, although in one case 30 cc. gradually increased to 100 cc. of a bouillon culture were introduced each day for ten days directly into the stomach of a rabbit by means of a stomach tube. The virulence of the organism used may of course have much to do with the success of such experiments.

By injecting 1.5 cc. of a thirty-hour-old bouillon culture of the typhoid bacillus into the peritoneal cavity of a guinea-pig and gradually increasing the amount every day up to 3 cc. on the fifth day, there were obtained on the sixth day an enlarged spleen in which were many large cells filled with red blood globules, and a number of small yellowish foci irregularly distributed in the liver.

Rabbits and guinea-pigs are, however, poor animals in which to study the possible effects of the typhoid toxine for the reason that under what must be considered normal circumstances many phagocytic cells containing the more or less digested remains of lymphoid cells are present, often in abundance, in the lymphoid tissue of the small and large intestine. Attention was first called to these cells by Flemming. Besides these lesions in the intestine, pigmented cells in the mesenteric lymph glands and spleen, and small areas of necrosis in the liver are not infrequent. They are entirely distinct from lesions caused by coccidia, and strongly suggest an origin due to absorption of toxic substances from the intestinal tract. The lesions in the liver are probably embolic in origin, but focal lesions in the liver of these animals are much more difficult to fathom, partly because the capillaries are very narrow and easily occluded, but principally because it is difficult to get perfect preservation of the various cell elements and of the red blood globules, a difficulty due in part at least to the large amount of glycogen present in the liver cells.

CONCLUSIONS.

The typhoid bacillus produces a mild diffusible toxine, partly within the intestinal tract, partly within the blood and organs of the body.

This toxine produces proliferation of endothelial cells which acquire for a certain length of time malignant properties.

The new-formed cells are epithelioid in character, have irregular, lightly staining, eccentrically situated nuclei, abundant, sharply defined, acidophilic protoplasm, and are characterized by marked phagocytic properties.

These phagocytic cells are produced most abundantly along the line of absorption from the intestinal tract, both in the lymphatic apparatus and in the blood-vessels.

They are also produced by distribution of the toxine through the general circulation, in greatest numbers where the circulation is slowest.

Finally, they are produced all over the body in the lymphatic spaces and vessels by absorption of the toxine eliminated from the blood-vessels.

The swelling of the intestinal lymphoid tissue of the mesenteric lymph nodes, and of the spleen is due almost entirely to the formation of phagocytic cells.

The necrosis of the intestinal lymphoid tissue is accidental in nature and is caused through occlusion of the veins and capillaries by fibrinous thrombi, which owe their origin to degeneration of phagocytic cells beneath the lining endothelium of the vessels.

Two varieties of focal lesions occur in the liver: one consists of the formation of phagocytic cells in the lymph spaces and vessels around the portal vessels under the action of the toxine absorbed by the lymphatics; the other is due to obstruction of liver capillaries by phagocytic cells derived in small part from the lining endothelium of the liver capillaries, but chiefly by embolism through the portal circulation of cells originating from the endothelium of the blood-vessels of the intestine and spleen. The liver cells lying between the occluded capillaries undergo necrosis and disappear. Later the foci of cells degenerate and fibrin forms between them. Invasion with polymorphonuclear leucocytes is rare.

Many of the phagocytic cells pass through the liver and lungs, and get into the general circulation. A few come from the abdominal lymphatics through the thoracic duct.

Focal lesions may arise in the kidneys by occlusion of the veins of the pyramids by emboli of these phagocytic cells.

Focal collections of phagocytic cells may occur in the heart and testicle by occlusion of lymph vessels.

The various sequelæ of typhoid fever deserve more careful bacteriological and histological examination, as shown by the study in one case of abscesses of the spleen where these seemed to arise in previously necrotic tissue, and of a case of pneumonia due to the pneumococcus, but complicated by the presence of the typhoid bacillus, in which great numbers of phagocytic cells were found in the exudation.

The thrombi which occur in the heart and in the veins of the lower extremities in the course of typhoid fever probably owe their origin to the same sort of lesions that cause occlusion of the vessels in the intestine.

Histologically the typhoid process is proliferative and stands in close relationship to tuberculosis, but the lesions are diffuse and bear no intimate relation to the typhoid bacillus, while the tubercular process is focal and stands in the closest relation to the tubercle bacillus.

BIBLIOGRAPHY.

Billroth.—Virchow's *Archiv,* xxi.

——. Virchow's *Archiv,* xxiii.

Eichhorst.—*Deutsches Archiv f. klin. Med.,* xiv.

Flemming.—*Archiv f. mikr. Anat.,* xxii.

Friedreich.—Virchow's *Archiv,* xii.

Grohe.—Virchow's *Archiv,* xx.

Hoffmann.—Untersuchungen über die pathologisch-anatomischen Veränderungen der Organe beim Abdominaltyphus, 1869.

Klein.—Report on the Intimate Anatomical Changes in Enteric or Typhoid Fever, 1875.

Orth.—Lehrbuch der speciellen pathologischen Anatomie, 1887.

Reed.—*Johns Hopkins Hospital Reports,* 1895.

Remlinger.—*Annales de l'Institut Pasteur,* 1897.

Ribbert.—Lehrbuch der pathologischen Histologie, 1896.

Rokitansky.—Pathologische Anatomie, 1861.

Virchow.—Virchow's *Archiv,* lii.

Wagner.—*Archiv f. Heilkunde,* i.

——. *Archiv f. Heilkunde,* ii.

DESCRIPTION OF PLATES LIV-LXI.

PLATE LIV.

Fig. 1.—Lymph nodule and adjoining tissue from a patch of Peyer showing the centre of the nodule occupied by large phagocytic cells.

Fig. 2.—A solitary lymph nodule showing diffuse formation of phagocytic cells.

PLATE LV.

Fig. 3.—Mucous membrane of intestine showing diffuse formation of phagocytic cells; also a dilated lacteal filled with phagocytic cells.

Figs. 4, 5 and 6 show details from Fig. 1.

Fig. 4.—From periphery of lymph nodule, showing a large cell of the reticulum in mitosis.

Fig. 5.—From near the periphery of lymph nodule, showing newly-formed cells beginning to incorporate the lymphoid cells.

Fig. 6.—From centre of lymph nodule, showing the newly-formed cells filled with more or less digested lymphoid cells. Several red blood globules have also been incorporated.

Fig. 7.—Lymphatic vessel of mucous membrane containing phagocytic, lymphoid and plasma cells.

PLATE LVI.

Fig. 8.—Terminal lymphatic (lacteal) of mucous membrane dilated and filled with phagocytic cells.

Fig. 9.—Mitosis of endothelial cell of capillary in mucous membrane.

Fig. 10.—Phagocytic cells in large vein of submucosa.

Fig. 11.—Plasma cell in mitosis inside phagocytic cell in a large vein in submucosa.

Fig. 12.—Mitosis of endothelial cell lining large vein in submucosa.

Fig. 13.—Giant cell in submucosa.

Fig. 14.—Two plasma cells migrating through wall of vein in submucosa.

PLATE LVII.

Fig. 15.—Endothelium of vein shoved forward by a swollen plasma cell, two lymphoid and two large cells; the last are degenerating.

Fig. 16.—Another section of the same vessel showing fibrin forming around large, degenerated cells.

Fig. 17.—Vein occluded by a mass of fibrin.

Fig. 18.—Large phagocytic cells beneath endocardium of heart.

Fig. 19.—Mass of fibrin and degenerating cells beneath lining endothelium of a large vein in the submucosa. Mitosis of large cell at one end.

PLATE LVIII.

Fig. 20.—Mitosis of endothelial cell in capillary of liver.

Fig. 21.—Phagocytic cells in a portal vein in the liver.

Fig. 22.—Phagocytic cells occluding capillaries in the liver.

Fig. 23.—Mitosis of a large phagocytic cell containing a red blood globule in portal vein in liver.

Fig. 24.—A giant cell formed apparently in a capillary in the liver.

Fig. 25.—Phagocytic cells occluding capillaries of liver. Beginning necrosis of included liver cells.

PLATE LIX.

Fig. 26.—Focal area of phagocytic cells in capillaries of liver showing beginning degeneration with formation of fibrin.

Fig. 27.—Focal area in liver due to phagocytic cells in the capillaries. Necrosis of many of the included and adjoining liver cells.

PLATE LX.

Fig. 28.—Phagocytic cells undergoing necrosis in capillaries between trabeculæ of liver cells.

Fig. 29.—Large focal area in liver consisting of phagocytic cells. The included liver cells have entirely disappeared.

PLATE LXI.

Fig. 30.—Large phagocytic cell in renal vein: judging from its inclusions it probably reached the general circulation through the thoracic duct.

Fig. 31.—Phagocytic cells in the exudation in the lung; from a case of lobar pneumonia complicating typhoid fever.

Fig. 32.—Group of cells in clot from left side of heart.

Fig. 33.—Phagocytic cells in a pulmonary blood-vessel.

Fig. 34.—Lining endothelium of large vein of spleen lifted up by phagocytic, lymphoid and plasma cells.

FIG. 1.

FIG. 2.

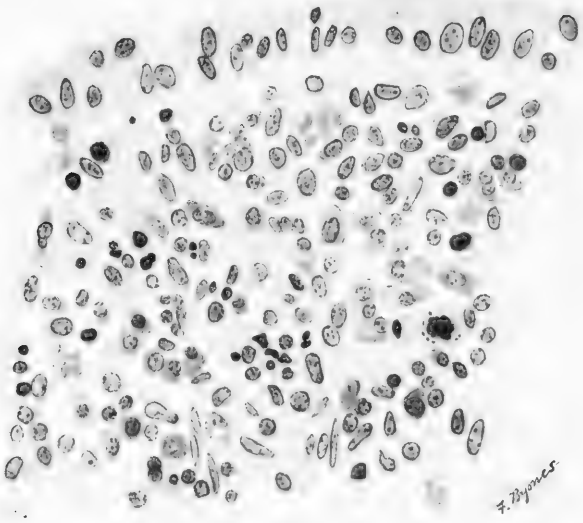


FIG. 3.

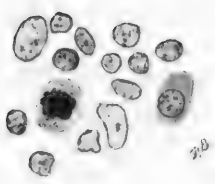


FIG. 4.

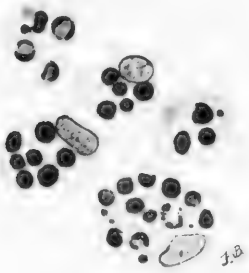


FIG. 6.

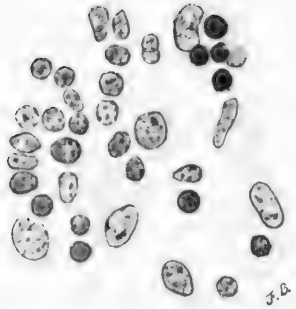


FIG. 5.

FIG. 7.

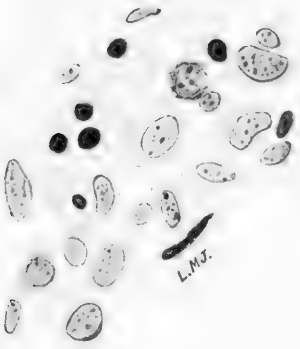


FIG. 8.

FIG. 12.

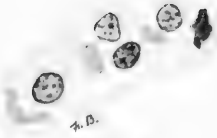


FIG. 9.

FIG. 11.

FIG. 13.

FIG. 10.

FIG. 14.

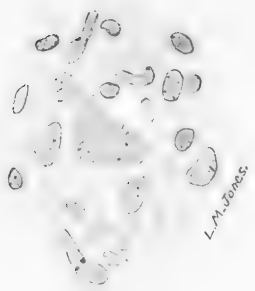


FIG. 15.

FIG. 17.

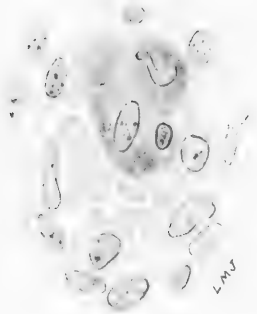


FIG. 16.

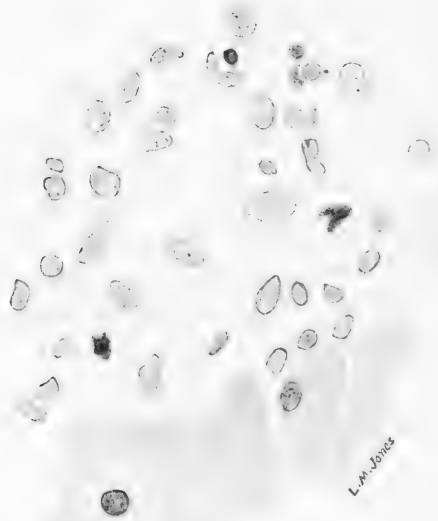


FIG. 18.

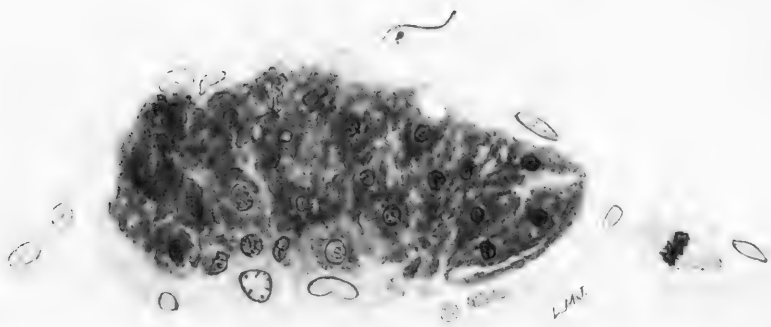


FIG. 19.

FIG. 20.

FIG. 23.

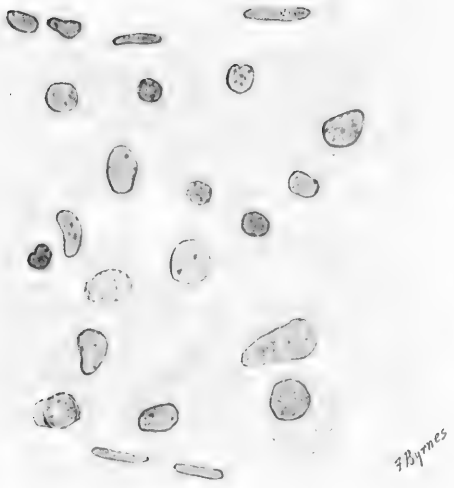


FIG. 21.

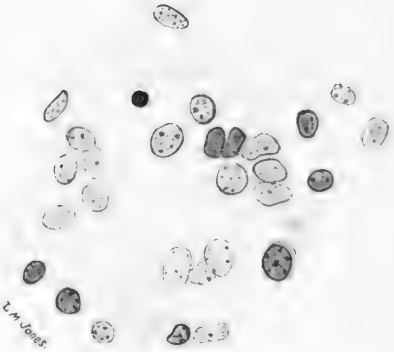


FIG. 24.

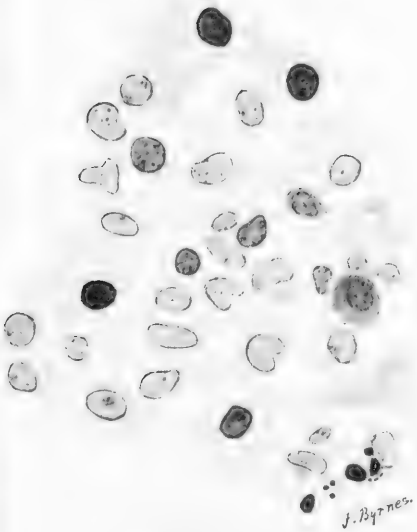


FIG. 22.

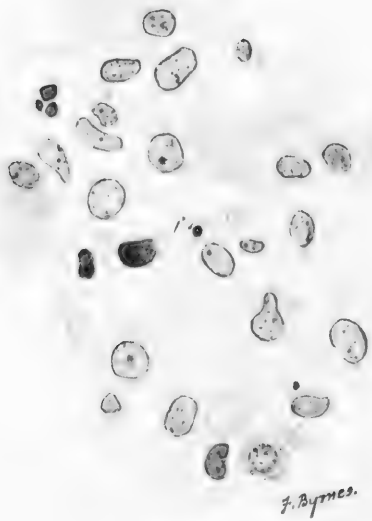


FIG. 25.

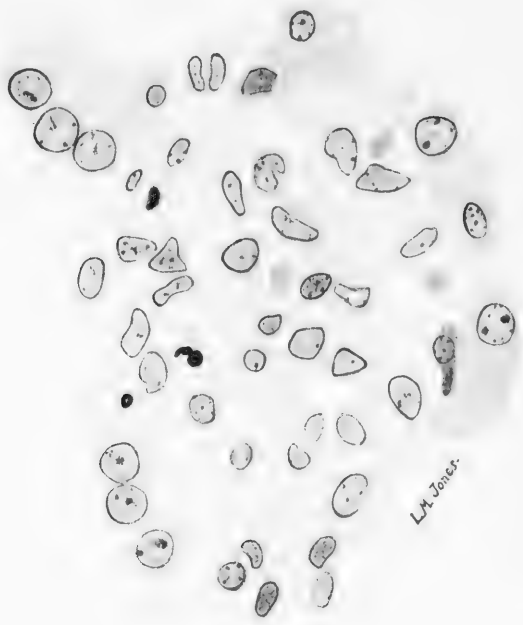


FIG. 26.

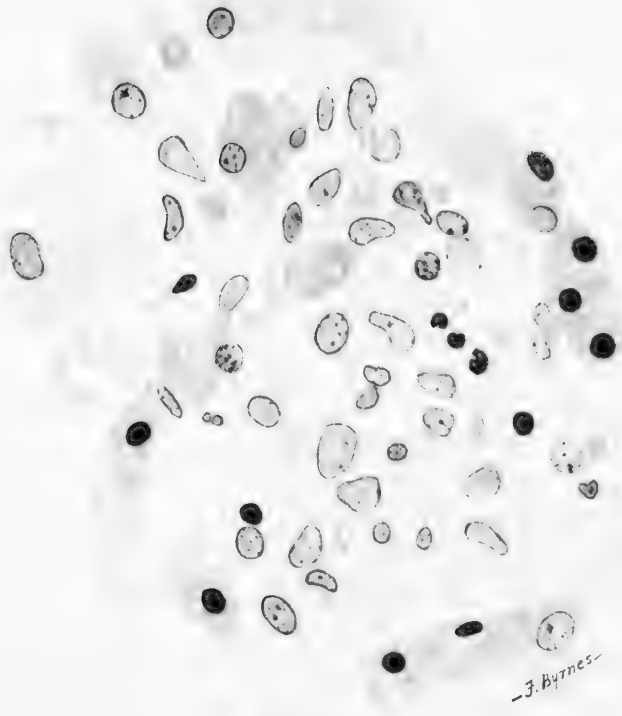


FIG. 27.

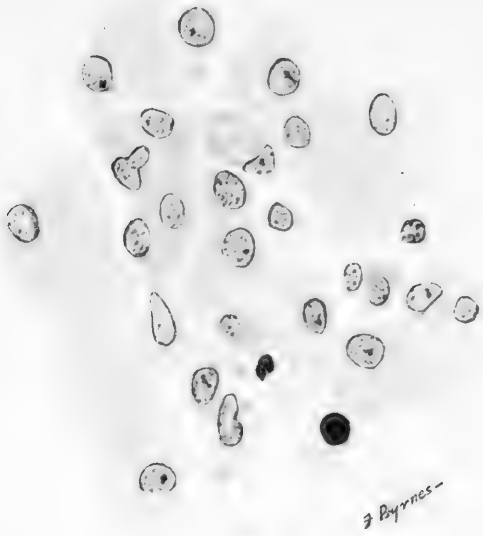


FIG. 28.

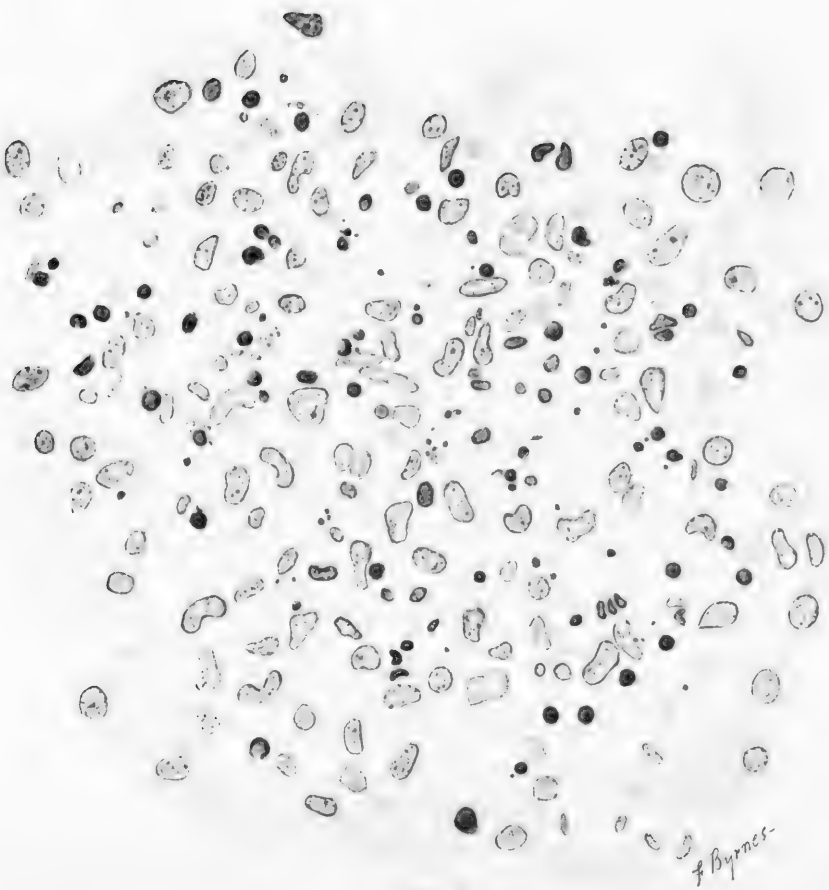


FIG. 29.

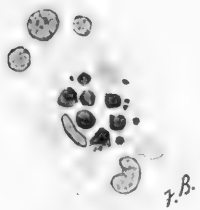


FIG. 30.

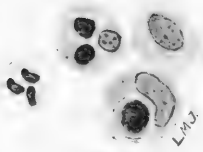


FIG. 32.

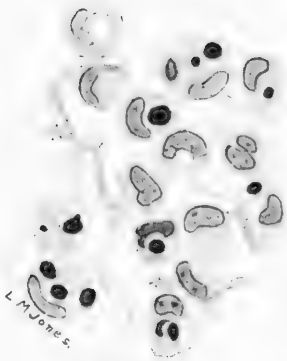


FIG. 31.

FIG. 33.

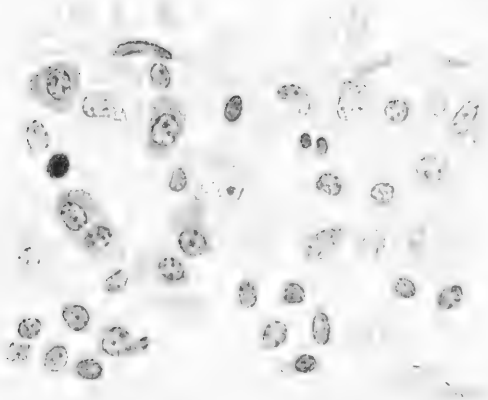


FIG. 34.

THE LEUCOCYTOSIS OF LABOR AND THE PUERPERIUM.

By CLEON M. HIBBARD, A. M., M. D., and FRANKLIN W. WHITE, S. B., M. D., of Boston.

The first investigators to find a leucocytosis in pregnancy were Moleschott and Nasse (1). This observation has been confirmed by Mallassez (2), Halla (3), Fouassier (4), Virchow (5), Krüger (6), Kosina and Eckert (7), Limbeck (8), Rieder (9), and others. It has been found that most primiparæ have a leucocytosis in the later months of pregnancy, the average count being about 13,000. About 5 per cent of multiparæ have a leucocytosis, usually less high than the primiparæ. Virchow suggests as an explanation of the leucocytosis of pregnancy the gradual increase in size of the lymph vessels and glands about the uterus which occurs at this time, which then send a fresh supply of corpuscles into the blood. Rieder says there is no digestion leucocytosis in pregnancy.

A smaller number of observations have been made to determine the leucocytosis during and after labor. Kosina and Eckert found in 16 cases during labor 10,540 to 18,600 leucocytes per cub. mm. The count sometimes increased a little after delivery, but more often remained the same, and from the first day of convalescence gradually fell to normal. Krüger places the average in labor cases at about 13,000 leucocytes per cub. mm. Mallassez speaks of a rapid increase of leucocytes after delivery, followed by a gradual decrease to normal during convalescence. Rieder says the leucocytosis directly before and after birth is about two or three times the normal (7700) and falls rapidly from that time to the end of the puerperium. Cabot (10) counted 12 cases, 9 primiparæ and 3 multiparæ, during and after labor; the leucocytes varied from 10,000 to 37,000 in primiparæ, and from 11,000 to 16,000 in multiparæ. There was a rapid fall in count after confinement. Concerning the kind of white corpuscles causing the leucocytosis in pregnancy, Björkman (11) says, "In the last stages

of pregnancy we find an increase of the white mononuclear elements very manifest and constant in primiparæ." Rieder gives differential counts of two cases, one multipara and one primipara, with a leucocytosis of about 13,000, and says this is not due to an increased number of polynuclear cells. The different kinds of cells in these cases preserved practically their normal relations.

In puerperal sepsis Limbeck found no leucocytosis present. Kosina and Eckert say that in puerperal fever the count rises in proportion to its severity, and after the fever is over again falls. Rieder, Grawitz (12), Kanthack (13), and others, find a leucocytosis present. Cabot says a leucocytosis is absent in severe and mild cases, and present in those of moderate severity.

Limbeck affirms that the effect of lactation on leucocytosis is not known. He suggests that the increased consumption of food in lactation may increase it.

In the past year Dr. W. L. Richardson, visiting physician to the Boston Lying-in Hospital, kindly gave us the opportunity of making the following observations during his service. The blood of 55 hospital patients was examined with reference to the number and kind of white corpuscles present. Our object was to determine the amount of leucocytosis present in labor and in normal convalescence after confinement, the peculiarities due to the age of the patient, and the beginning of lactation; also the effect of hæmorrhage, prolonged labor, septicæmia and inflammation of the breasts in causing departures from the normal. It was hoped that in doubtful cases of septicæmia and suppuration of the breasts the blood count might be a help to early diagnosis.

The blood was examined in the 24 hours preceding delivery, usually in the first stage of labor; also on the 1st, 3rd, 5th, 7th, 10th and 13th days after confinement (see Table I). Thirty-three of the patients were primiparæ, 22 multiparæ, the ages varying from 16 to 41 years. Taking 10,000 leucocytes per cub. mm. as the maximum normal, a leucocytosis was found to be present before delivery in 84 per cent of the primiparæ and 75 per cent of the multiparæ, the average for 32 primiparæ being 15,021, an increase of 50 per cent above the normal,

Case No.	Pregnancy.	Age.	Stage of labor.	Time of count before delivery.	Duration of labor in hours.	Hæmorrhage.[1]	Temperature.[2]	Count before delivery.	Days of convalescence. 1st.	2nd.	3rd.	4th.	5th.	6th.	7th.
1	I	21	1st	5.20	11	Sl.	T.	9,600	14,380	15,000		10,100		14,800	
2	I	21	2nd	2.00	(?)	N.	T.	14,500	13,600		11,200		12,800	15,500	9,400
3	I	18	2nd	0.15	6	N.		14,400	11,700		7,000		9,400		9,800
4	I	24	1st	1.30	14	N.	T.	15,000	8,600	19,060	19,300		12,800		7,200
5	I	23	1st	7.00	12	C.		16,800	17,200		11,730		9,200		10,000
6	I	23	1st	6.30	37	N.	T.	7,600	17,200		13,400		13,200		14,200
7	I	22	1st	2.30	8	Sl.		14,200	9,800		8,000		7,200		6,800
8	I	22	1st	18.20	37	N.		11,400	10,500		11,600		8,200		6,100
9	I	23	2nd	0.30	6	Sl.		21,200	7,800		6,300		7,000		7,400
10	I	23	1st	7.00	20	Sl.		10,200	6,400		6,400		7,500		8,800
11	I	22			108	Sl.		13,000	8,800		6,500				12,200
12	I	20	1st	7.20	20	N.		15,600		6,900	8,200		7,500		
13	I	16	2nd	1.10	22	N.		21,000	13,500		8,100		12,000		
14	I	21	2nd	2.25	42	N.		30,600	19,400		10,400		10,100		
15	I	26	1st	5.00	53	C.	T.	14,100	18,600		9,600		13,600		10,800
16	I	25	1st	4.00	17	N.		24,300	24,000			11,700			11,200
17	I	17	2nd	0.15	25	N.		16,800	20,000			9,400			12,800
18	I	18	1st	9.00	18	N.		8,100	9,800		11,600		9,600		9,200
19	I	17	1st	4.50	33	N.		18,400	15,600		9,400		8,800		9,500
20	I	22	2nd	2.00	12	N.	T.	12,600	10,600				8,600		
21	I	25	2nd	0.10	8	Sl.		21,900	15,300		11,900		8,000		8,800
22	I	21	1st	7.00	19	C.	T.	14,500	13,600	7,800	9,100		9,000		9,600
23	I	21			20	N.		17,000	8,400		13,600		5,600		7,800
24	I	25			21	N.		19,400			8,300				12,000
25	I	17	1st	12.00	11	N.		9,800			10,300				14,000
26	I	23	1st	7.00	24	Sl.		11,200	7,800		10,000		7,300		8,400
27	I	17			3	N.		12,200	14,000		14,000		11,600		9,600
28	I	30	1st	27.00	41	N.		12,200	12,300		9,100		8,200		12,100
29	I	32	2nd	1.40	45	N.		15,500	12,000				7,600		10,000
30	I	27	1st	4.00	12	N.	T.	8,000	9,400		5,900		8,400		8,400
31	I	18			(?)	N.	T.	17,700	21,300						
32	I	17	1st	7.00	10	N.	T.	11,900	20,200		14,000		19,000		17,200
33	I	20			31	P.P.	T.		23,300			11,800		9,400	
34	II	22	2nd	0.25	7	Sl.		8,400	9,900		8,600		9,100		9,000
35	II	22	1st	1.00	8	C.		12,000	8,200		11,000		15,200		19,300
36	II	19	1st	5.15	30	Sl.		8,000	9,300		6,000		9,400		7,700
37	II	24			7	C.		10,100	10,600		7,600		6,100		6,000
38	II	26	1st	1.45	9	Sl.		10,200	9,600		7,200				11,100
39	II	30	2nd	0.50	6	P.P.		9,900	12,300		12,000		11,200		
40	II	23			12	N.		20,500		10,000			6,600		7,000
41	II	22	2nd	1.00	4	N.		14,200	6,000		12,800		10,400		12,300
42	II	32	1st	3.00	8	N.		12,500	12,100		12,700		9,600		11,800
43	III	33	1st	2.00	12	Sl.		15,700	12,300		12,000		9,400		8,600
44	III	27	1st	1.00	7	N.		13,200	7,200		6,800		6,800		10,500
45	III	26	2nd	0.55	9	N.		13,300	12,400		8,400		8,400		10,400
46	III	23	1st	1.00	8	Sl.		11,100	12,300		7,600		10,000		10,000
47	III	30	2nd	1.30	33	C.		8,000	8,800		3,000		5,500		
48	III	25	1st	1.20	10	C.	T.	12,800	16,100		8,800				8,200
49	III	30	1st	3.00	(?)	N.	T.	10,200	11,200		10,100		7,000		
50	IV	39	1st	1.50	11	C.		17,200	10,800		8,000		5,900		10,600
51	IV	22	1st	1.30	4	N.		10,600	10,300		6,900		5,800		
52	VII	34	1st	5.00	10	N.		10,500	6,000		5,700		5,900		6,200
53	IX	33			8	N.		8,600	8,300		6,000		6,900		4,100
54	IX	35			6	C.			19,200		7,000		7,200		7,600
55	IX	41			11	N.	T.			18,000		8,700			

[1] Hæmorrhage in delivery Sl. = slight, N. = normal, C. = considerable, P. P. = post-partum hæmorrh

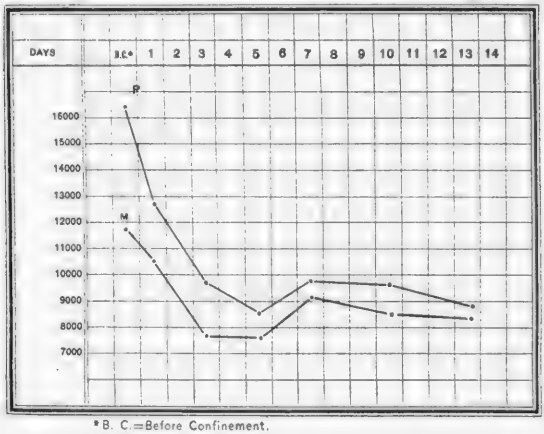
I.

9th.	10th.	11th.	12th.	13th.	REMARKS.
		13,200			Macerated foetus.
	7,600			2,900	15th, 10,900; 16th, 19,300; 17th, 10,000. Inflammation of breast on 5th and 16th days.
	10,600			8,000	
	12,500			15,000	Inflammation of breast on 8th to 10th day.
	8,000				
	9,400			7,600	Sepsis.
	10,400			7,100	
	7,000			10,000	
	7,400			6,100	
	8,600				
	7,000			9,000	17th, 7,200.
	9,200			9,500	
	14,200			13,100	
	10,100			7,600	Inflammation of breast on 4th to 7th day.
	9,000				
	8,800				
	12,000			13,200	
	17,200			11,700	
	9,000			11,000	Temp. 101° on the 13th day, cause not known.
	10,600				14th, 9,400.
		7,400		5,000	Sepsis.
	6,100			9,400	
	9,200				
	11,700			11,500	
	10,600			8,400	
	10,300			6,900	
				8,200	
	11,200			6,200	
	7,800		10,300	6,000	Inflammation of breast on 12th to 15th day.
					Eclampsia.
	16,300		17,900		Anaemia, petit mal.
10,300					Red corpuscles on 1st day, 1,500,000.
	7,400				
	10,800			13,200	
	9,800				
	10,700				
	4,800				
9,500				8,000	
	6,400				
	7,600				
	12,700				
	8,200			8,800	
	6,600				
	8,000				
	6,300	10,000		9,400	
	6,400			6,200	
	13,400				Alveolar inflammation on 10th day.
	5,500				Macerated foetus.
	16,800				
	8,300			6,800	
	6,700			5,500	
	9,400				Placenta praevia.
	6,600			9,800	Sepsis.

[2] Cases marked T. had a temperature over 100° during convalescence.

the average for 20 multiparæ being 11,700, an increase of 17 per cent. In 22 primiparæ and 17 multiparæ, the convalescence was perfectly normal and afebrile, and the average of the blood counts in these cases is taken to represent the normal leucocytosis of convalescence. The average for these primiparæ before delivery was 16,100, and for the multiparæ 11,800. The amount of the leucocytosis fell rapidly after delivery, reaching normal about the 4th or 5th day, then rose slightly till the 7th day, after which it gradually fell again to normal. This is graphically represented on the following chart (see Chart A). The curves for primiparæ and multiparæ show the same characteristics

CHART A.



* B. C.=Before Confinement.

throughout, the number of leucocytes in the multiparæ being less. The disturbances in the breasts which commonly accompany the beginning of lactation, namely, swelling, induration and tenderness, are at their maximum from the 3rd to the 6th day after delivery, and it is interesting no note that the increase in the leucocytosis immediately follows this disturbance. We are inclined to regard this change in the blood as a result of the changes in the breast accompanying lactation. In considering the effect of age, the primiparæ were divided into two groups, those 21 years and under, and those over 21 years of age; and it was found that the leucocytosis in the younger women was

above the average. The same was true of multiparæ divided into two equal groups, above and below 26 years of age.

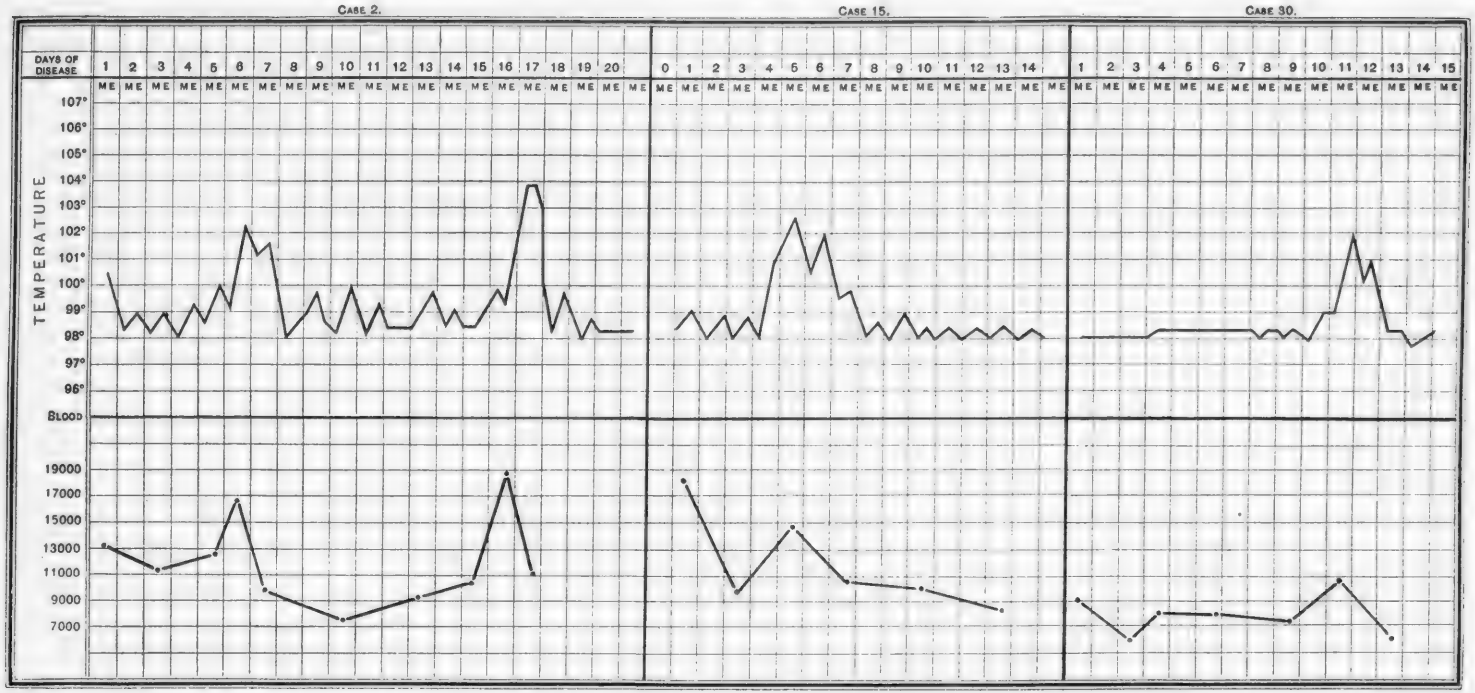
The variation in the leucocytosis early and late in labor was next considered. The counts were made at different times before delivery, and on dividing the counts in primiparæ into two equal parts, according as they were made within four hours of delivery or earlier, it was found that the early counts averaged 12,200 and the late ones 17,600. The multiparæ showed a similar but less marked contrast between the early and the late counts, 9300 and 12,00 respectively.

Effect of Haemorrhage.—The average blood counts of 5 multiparæ who lost considerably more than the usual amount of blood after delivery showed a leucocytosis about 1500 above the normal average the day after confinement. Two cases of severe post-partum hæmorrhage (Nos. 33 and 39; see table), one a primipara, the other a multipara, showed a leucocytosis of 3000 or 4000 above the normal average for the first five days after delivery, the worst case having 1,500,000 red corpuscles and 23,000 white corpuscles the day after confinement.

Inflammation of the Breast.—Four primiparæ (Nos. 2, 4, 15 and 30) during convalescence had an inflammation of the breast, accompanied with fever of about 102°, congestion and tenderness, and in two cases with a small definite red indurated area in the breast. The febrile attacks were all mild, lasting only two or three days, terminating in complete recovery and without any indication of the presence of pus. In all these cases there was a marked increase of the leucocytosis just at the time of the febrile attack, which disappeared, however, immediately after it, the maximum counts being from 11,000 to 19,000. This is graphically shown on the accompanying chart (Chart B). Case 2 had two separate attacks of mastitis on the 6th and 7th days, and on the 16th and 17th days of convalescence. Case 15 had mastitis from the 4th to the 7th day. Case 30 from the 12th to the 14th day. Further observation of these cases is desirable, but it would seem that inasmuch as the blood count is so strongly affected by mild cases of mastitis, it can be of little help in early diagnosis of the severe purulent form.

Only three cases of *septicaemia*, all of moderate severity, came under

CHART B.


Case 2.
Case 15.
Case 30.
DAYS OF DISEASE
ME
TEMPERATURE
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
Blood
19000
17000
15000
13000
11000
9000
7000

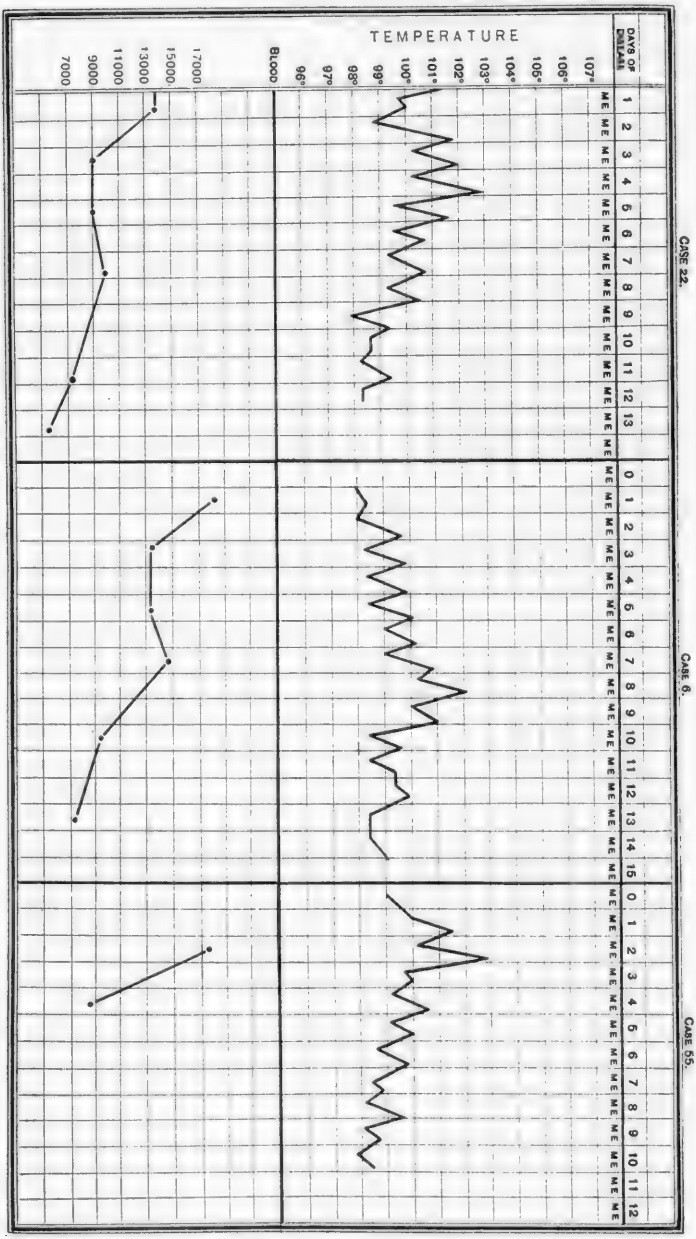


CHART C.

our observation. In Case 22 the leucocytosis ran a normal course; in Cases 6 and 55 there was a marked increase, the count varying from 14,000 to 17,000. When these patients had had the usual curetting and antiseptic treatment, and the fever and constitutional symptoms began to abate, the leucocytes subsided rapidly (see Chart C). Case 22 was curetted on the 3rd and 4th days; Case 6 on the 8th day; Case 55 on the 2nd day.

The septic cases are too few to serve as a basis for any conclusions.

It is interesting to note that in one fatal case of eclampsia (No. 31) the leucocytosis was 17,700 before delivery and 21,300 the following day.

TABLE II.

Case No.	Preg.	Leucocytosis.	Polynuclears.	Large Mononuclears.	Lymphocytes.	Eosinophiles.	Large Mononuclears. Absol. No. in 1 cmm.	Lymphocytes. Absolute No. in 1 cmm.
14	I	30600	93.2	1.8	4.4	0.6	552	1346
16	I	24300	90.	3.	6.	1.	729	1458
21	I	21900	95.8	1.2	3.	.0	263	657
9	I	21200	90.4	1.6	8.	0.8	339	1696
13	I	21000	95.2	2.6	2.	0.2	546	420
40	II	20500	91.	4.	4.	1.0	820	854
24	I	19400	94.2	4.4	4.4	.0	272	820
19	I	18400	86.2	2.6	10.	1.2	498	1840
5	I	17200	92.	3.	4.	1.	516	688
50	IV	17200	79.6	2.	16.8	2.6	344	2890
17	I	16800	89.	4.2	6.2	0.6	706	1042
4	I	15000	73.	5.	21.	1.	750	3150
2	I	14500	93.	1.6	5.4	0.	232	573
11	I	13000	80.	4.	15.	1.	520	1950
48	III	12800	86.5	4.5	8.1	0.7	576	1037
53	IX	8600	74.6	6.6	17.4	1.4	567	1496
34	II	8100	76.	6.4	17.2	0.4	518	1393
30	I	8000	74.2	7.2	17.9	0.7	576	1432
47	III	8000	72.	8.	20.	0.	640	1600

A differential count of the white corpuscles was made in 19 cases in labor, 12 with rather high counts, 4 with normal counts for comparison, and 3 of intermediate grade (see Table II). In the four normal cases the proportion of the different white corpuscles was practically normal. In the other 15 cases, with one exception (Case 4), the leucocytosis consisted in a marked relative and absolute increase of the polynuclear cells, the percentage of these cells being greater, as a rule, the higher the leucocytosis. There is a relative and slight absolute

diminution in the number of lymphocytes, while the large mononuclear cells are relatively diminished and absolutely are normal.

Our results differ markedly from those of Rieder and Björkman. We will sum up our conclusions briefly as follows:

(1). A leucocytosis was present in over three-fourths of our cases in labor, being more frequent and higher in primiparæ.

(2). During convalescence the count falls rapidly at first, later more gradually, to normal. About the 7th day there is usually a slight rise.

(3). The leucocytosis is usually higher in the younger women, regardless of the number of the pregnancy.

(4). The patients farthest advanced in labor have the highest counts.

(5). Breast inflammation, even when mild, causes a prompt leucocytosis, hence the blood count has no value in the early diagnosis of breast abscess.

(6). The leucocytosis present at the time of labor is due to an increase of the polynuclear cells.

BIBLIOGRAPHY.

1. Moleschott and Nasse.—Die Zählung d. Blutkörperchen u. deren Bedeutung f. d. Diagnose u. Therapie, Leipzig, 1891.

2. Mallassez.—*Archiv de physiol.*, 1874, p. 22.

3. Halle.—*Zeitschr. f. Heilk.*, 1883, iv, p. 199.

4. Fouassier.—Thèse de Paris, 1886.

5. Virchow.—Cellularpathologie, 4 Aufl., 1871, p. 167.

6. Krüger.—Virchow's *Archiv*, cvi, p. 1.

7. Kosina and Eckert.—Hoffman and Schwalbe's *Jahresbericht*, 1881, x, Abth. 2, p. 158.

8. Limbeck.—Grundriss der klin. Pathol. d. Blutes, 1896.

9. Rieder.—Beiträge zur Kenntniss der Leucocytosis, 1892.

10. Cabot.—Clinical Examination of Blood, 1897.

11. Björkman.—*Amer. Medico-Surgical Bulletin*, p. 17, 1894.

12. Grawitz.—Klin. Pathol. d. Blutes, 1896.

13. Kanthack.—*Brit. Med. Journal*, June, 1892.

ONE OF THE CONDITIONS UNDER WHICH DISCONTINUOUS STERILIZATION MAY BE INEFFECTIVE.

By THEOBALD SMITH, M. D.

(*From the Laboratory of Comparative Pathology, Harvard Medical School.*)

Since the process of sterilization by heat forms the foundation for all bacteriological work, we might take it for granted that this subject had been pretty well exhausted. New phases, however, appear with new kinds of work, and the discontinuous sterilization of large quantities of bouillon has been so unsatisfactory in my hands that I deem it worth while to present some facts upon this well-worn subject. Without doubt experiences similar to those related below have visited other laboratories, but thus far the causes have not been discussed. Text-books are, as a rule, silent upon the subject. Discontinuous heating at 100° C. is regarded as co-ordinate and interchangeable with the use of a more elevated temperature, and the whole subject is dismissed very briefly. Among a large number of text-books examined Abbott's Principles of Bacteriology was the only one found in which some qualifying statement is made in regard to this matter: "It must be borne in mind that this method of sterilization is only applicable in those cases which present conditions favorable to the germination of the spores into mature vegetative cells. Dry substances or organic materials in which decomposition is far advanced, where the conditions of nutrition favorable to the germination of spores are not present, cannot be successfully sterilized by the intermittent method."*

The general proposition as here stated includes the subject I wish to discuss. The illustrations given do not, however, strictly apply to the bacteriological laboratory, since dry or decomposed substances are rarely dealt with there. A more apt illustration is the condition to be described.

* Second edition, p. 53.

For more than a year past bouillon steamed in shallow layers (2 to 3 cm. deep) in the Arnold sterilizer for an hour on 3 or 4 successive days, though remaining indefinitely clear if unused, showed after some days' growth of diphtheria bacilli the presence of anaërobes. The source of the contamination was at first looked for in some error of manipulation during inoculation and in the stock culture employed. Soon, however, both these sources were excluded and the infection was referred to spores which had survived the boiling. It is interesting to note here that the bouillon became favorable to the germination of these spores and the multiplication of the bacilli only after the diphtheria bacilli had formed a membrane on the surface and were appropriating all the available oxygen. None of the flasks were ever found harboring aërobic spore-bearing bacilli.

Similar favoring conditions have been observed in the past on the surface of solid culture media when aërobes and anaërobes were introduced together. That the former may arouse slumbering spores of the latter lurking in culture media has not, I believe, been suggested, although the matter is self-evident. This experience is, as it were, an echo of the prolonged controversy which raged over the hypothesis of abiogenesis. Even at this day the acuteness of the intellects that finally solved the problem by satisfactory demonstrations is forcibly impressed upon the stumbling experimenter. In the case before us the conditions are somewhat different from those formulated by the participants in this controversy. We have a "putrescible" fluid which remains limpid. Inoculated with one bacillus, it apparently gives rise to another wholly different organism. The constancy of the second form in all the flasks examined, a large bacillus with a terminal spore, might in a more primitive state of our knowledge concerning bacteria have led to seemingly reasonable speculations concerning "the mutation of species" and the "spore-bearing phase of the diphtheria bacillus."

Discontinuous sterilization cannot therefore be relied upon when shallow layers of fluid freely exposed to the air are to be freed of bacteria. The spores of anaërobes accidentally present cannot find the conditions favorable to their germination in the intervals between

the successive applications of heat. The autoclave is therefore indicated, for a temperature of 110° to 115° C. definitely rids the fluid of them. In short, a bacteriological laboratory would be seriously crippled without apparatus furnishing temperatures above the boiling point. But by a round-about method discontinuous sterilization may be even here made successful.

Having cultivated anaërobes for a number of years without the use of hydrogen in vessels having a restricted communication with the air, I filled round litre flasks with bouillon to the neck and had them steamed three of four times and then placed in the incubator. In several instances they became suddenly turbid after 2 days by reason of the almost explosive rapidity with which anaërobic bacilli multiplied, thus proving the theory of imperfect sterilization of the bouillon used for the cultivation of diphtheria bacilli. Most of the flasks remained sterile after 4 steamings with intervals of incubation between them gradually lengthened to 48 hours. It is not to be denied that there may be still other anaërobes whose sensitiveness to aërobic conditions is so great that development may not take place even in the nearly full round flasks. In several instances bouillon prepared for the cultivation of tetanus bacilli became clouded with anaërobes after four or five steamings, indicating that even under favorable conditions spores of this character germinate with difficulty, and that not less than 48 hours of incubation should decide the sterility of the fluid. It is thus possible to eliminate anaërobes without the autoclave. If the bouillon is to be used in shallow layers, it must finally be poured or siphoned under suitable protection from the round flasks in which the sterilization was effected into the sterile culture flasks, but this round-about process will rarely be needed, since it does not appear that 110° to 115° C. is any more harmful than 100° C. to the nutritive quality of the bouillon.

This subject is of some importance in other respects. It is possible that now and then anaërobes present in tubes of milk, for instance, may remain dormant until some other organism is introduced. Subsequent changes in the milk due to the anaërobe may be ascribed to the organism introduced unless the sediment be carefully scrutinized

with the microscope. In tubes containing agar, gelatine, or other solid culture media, the restricted supply of oxygen in the bottom of the tubes will favor spore germination, so that the danger of subsequent contamination even when the autoclave is not resorted to is not likely to cause any trouble.

Sterilization from the point of view developed in these pages consists essentially in the destruction of all those bacteria or their spores which may subsequently find favorable conditions for development. The discovery by Globig* of very resistant spore forms and of bacteria which multiply only at a certain elevated temperature makes this definition accord with the facts. Inasmuch as our only test for the presence of life in any culture medium is its increase until visible mainly to the unaided eye, we are unable to affirm the non-existence of any life at all, although for practical purposes we may safely take it for granted after anaërobes have been destroyed.

The spores of adventitious anaërobes will probably be regarded as coming from contact of the beef with fecal matter at the abattoir—dust, dirt and utensils. There is, however, another source to which my attention has been called. In adding bits of tissue as large as peas from the organs of chloroformed rabbits and guinea-pigs, and in one case from the kidney of a pig slaughtered for food, to gelatine in tubes or to fermentation tubes pure cultures of anaërobes were obtained in four cases. It is thus not improbable that beef may contain a few stray spores which during the life of the animal have been carried from the digestive tube into different parts of the body, but which cannot germinate under prevailing conditions.

The various cultures thus far obtained from insufficiently sterilized bouillon and from animals have been reserved for further study and for possible identification with described forms. At present I may simply state that while they are all gas-producing bacilli bearing terminal spores, they evidently belong to different species or varieties of species, if we are to judge from the quite different odors and the variations in the gas formula $\frac{H}{CO_2}$ in dextrose bouillon as roughly determined in the fermentation tube.

* *Zeitschrift f. Hygiene*, iii (1887), 294, 322.

NOTES UPON AN EPIDEMIC OF FOWL CHOLERA AND UPON THE COMPARATIVE PRODUCTION OF ACID BY ALLIED BACTERIA.

By CHARLES H. HIGGINS, B. S., D. V. S.

(From the Molson Pathological Laboratory of McGill University.)

The bacillus of fowl cholera, although previously observed by Perroncito and others, was first successfully cultivated in 1880 by Pasteur, whose investigations of this organism mark an epoch in the bacteriological study of disease. Although this bacillus is well known and has been fully described, it has not been positively identified as the causal factor of disease upon this continent. Cholera in poultry, it is true, exists here, and in Bulletin No. 8 of the Bureau of Animal Industry there is to be found a very careful study of "Three Outbreaks of Fowl Cholera" by Dr. V. A. Moore. These epidemics occurred in places far apart, one near Providence, R. I., another in Virginia, and another near Washington. The germ from all three proved to be the same. It differed, however, in its characters from the European bacillus of fowl cholera, though closely allied thereto. The germ that I am about to describe would seem closely to resemble, if not to be identical with, the true bacillus of fowl cholera as found in various European countries.

The fact, then, that I have been able to isolate a germ which, while closely related to that causing outbreaks of a very similar nature along the Atlantic coast, has been found after prolonged study to be distinct from this form, would seem to be sufficient ground for publishing a description of the organism and its characters. In studying this form I have been enabled to pursue a series of investigations upon certain methods for the differentiation of species. Although I cannot state that these studies have led to complete success, it will, I think, be well to mention those employed by me, so that others may

know how much has been done along these lines and with how much success, and may possibly carry on the work to a more successful conclusion.

I have more especially attempted to arrive at methods for the study of the comparative acid production by allied forms of bacteria, employing germs of various virulence from this particular outbreak, together with forms isolated from the epidemics of fowl cholera in the States and cultures of the bacillus of the disease received from Europe. To gain a sure basis for such investigations I have endeavored to manufacture synthesized media so that I might more surely start from known compounds capable of being split up by bacterial growth. The number of synthesized media that I have produced* has been very great, but my success in the matter has only been partial, for the bacillus of fowl cholera and its allies grow but poorly upon nearly all the fluids made up by me. Nevertheless, I feel that even my partial success has given me fuller knowledge of this subject of acid production by bacteria, and the results obtained may possibly be deemed of value as throwing some light upon at least one more possible method of differentiating between closely allied forms.

My attention was called to this outbreak December 5, 1895, by Professor Adami, who had in turn heard of it through Dr. G. P. Girdwood. The outbreak occurred among a flock of fowls belonging to Mr. A., of St. Anne's, P. Q. St. Anne's is situated about 20 miles from the city of Montreal, on the island of Montreal, where the St. Lawrence divides to encircle the island. On the 8th I visited Mr. A., among whose fowls the outbreak had occurred.

History.—The first signs of the disease had been noticed about a month previously, the animals dying very suddenly. The disastrous results were communicated to Mrs. G., among whose fowls an outbreak had occurred several years previously. She suggested the placing of sulphuric acid and sulphate of iron in the drinking water and allowing access to no other water, also a complete disinfection of the hen-house. After cleansing the coop and administering the above remedy the disastrous results were rapidly checked, but few fowls having died since. The disease pursued a very rapid course, producing death in from 3 to 12 hours from the time that the first signs were noticed. The greater

* See addendum to this paper.

number were found dead in the morning (from two to four at a time). In all there were lost about 20 fowls, which were invariably among the youngest. The older ones seemed immune and none showed symptoms of the disease.

Symptoms.—From the outset there was a profuse diarrhœa, yellow in color; the feathers were ruffled and there was a yellowness about the head. The fowls were not inclined to move after noticeable symptoms occurred, but rather showed a tendency to roll themselves up in a ball, so to speak, and to remain motionless till they fell over dead.

The hygienic conditions were favorable for the development of any infectious disease. The fowls were confined in a coop about ten feet square and six feet high. There were two small windows, one on the east and the other on the west elevation, each being about two feet square. At the time of my visit the place was damp and was said to have been particularly so at the time of the outbreak. After the outbreak a fire was started in order to dry out the apartment. There was little or no ventilation.

Three animals dead of the disease in question were procured. The exact length of time they had been dead was not definitely known, but it was thought to have been at least a week. Upon the death of the fowls they were immediately thrown outside and were at the time of the visit covered with snow. For several days it had been very cold; there was a snow-storm on December 2nd and another the night before the visit (that of December 7th). To all appearances the fowls had been covered by both falls of snow. Probably they had been exposed during the time they had lain outside to a mean temperature of at least — 20° C., it having been very cold after the first fall of snow. On the night of the 8th they were left in a sleigh out of doors, a thermometer nearby registering —16° F. or —27° C. at 8 A. M. on December 9th. They were brought to the Molson Pathological Laboratory of McGill University on the morning of the 9th.

Morbid Anatomy.—The appearances found at the necropsy will be cited for but one case, as there was little or no difference noted in the three. The subject was a mongrel Plymouth Rock hen about eight months old. She was in good condition. The liver was rather pale with areas of necrosis. Spleen soft, pulpy and enlarged. The intestines were pale externally and free from evidence of hæmorrhage. Within, the mucosa was congested and swollen. The lower portion of the intestines was particularly affected, and its follicles were moderately distinct. There was fluid in the pericardial cavity, with some hæmorrhage beneath the

pericardium, and marked bloody imbibition of the vessel walls of various organs.

Cultures were made from various organs and fluids of the body, and revealed on the following day a pure and moderately vigorous growth of a non-motile bacillus. The bacillus in all the cultures from the same fowl appeared to be identical; the cultures from the various fowls were found to be identical and pure.

DESCRIPTION OF THE BACILLUS.

Morphology.—The micro-organism is a non-motile, rod-shaped organism, aerobic, though growing in the presence of little free oxygen, varying slightly in size according to the medium upon which it is grown. In broth at 37° C., at the end of 20 hours, the bacilli vary from 1.3 to 1.8 μ in length and from 0.5 to 0.7 μ in breadth. The most frequent form is a diplobacillus with rounded ends, which may under low magnification be mistaken for a diplococcus. In the hanging-drop preparation it shows a distinct polar arrangement of the protoplasm. It is frequently seen in old cultures in short chains consisting of three or four of the bacilli placed end to end. It stains with the ordinary aniline dyes but retains the coloring matter feebly or not at all when subjected to Gram's method. It appears in great numbers in the tissues and also in the blood. In the tissues it often occurs in clumps of four or five. No spores or any tendency to spore-formation have been recognized. A feebly-staining capsule has been occasionally observed.

Cultures in Broth.—This medium was made by using 2 grammes of Liebig's extract to 500 cc. of distilled water, 1 per cent Witte's peptone and 0.5 per cent rock salt. Kept at 37° C. the broth is uniformly clouded at the end of 24 hours. This cloudiness does not as a rule settle, but in some cases the broth eventually cleared, the conditions being kept unaltered. The germ was found to be still alive in tubes which had stayed in the laboratory for 9 weeks.

When ordinary broth is neutralized with hydrochloric acid or sodium hydrate, as the case may be, phenolphthalein being used as an indicator, the germ will grow between the limits of 4 per cent of a decinormal solution of hydrochloric acid and 4 per cent of a decinormal solution of sodium hydrate. Between these two limits the culture shows very little difference upon removal from the incubator at the end of 24 hours. Beyond these two points, on both the acid and alkaline sides, the growth is very feeble, and on tubes containing 8 per cent of decinormal hydrochloric acid and of those containing 10 per cent of decinormal sodium

hydrate solution there is no growth at the end of 24 hours. It may be added that there is a decided odor characteristic of broth cultures of this germ.

Fermentation (gas production) experiments by means of the Smith tubes proved negative with both 1 per cent lactose and 1 per cent glucose broth.

Nutrient Gelatine Cultures.—Here the Liebig's extract broth was used, 7 per cent of gelatine being added. This when made up was slightly acid (to phenolphthalein), but the acidity was well within the limits determined by the previous experiments with broth cultures.

The growth on the nutrient gelatine is less vigorous than in broth and is recognizable in stab cultures and on plates about the third or fourth day. In stab cultures there is little or no growth at the surface, the growth being equal all along the line of inoculation. On plates, superficial colonies about 0.5 mm. in diameter are seen on the third or fourth day; the deep colonies are more minute. The surface growths are granular without sharply-defined borders. The deep colonies show a granular appearance; their color is a very pale white. There is no liquefaction. It was noticeable that no cultures were obtained beneath mica plates. In other words the bacillus will not grow when deprived of free oxygen, but grows better where there is little oxygen (along the stab in stab cultures) than where oxygen is abundant (on the surface).

Agar Cultures.—The Liebig's extract broth was used for this purpose, 1 per cent of agar being added. This also was slightly acid to phenolphthalein, but the acidity was well within the limits of active growth. Growth on this medium at 37° C. is not very vigorous; at the end of 24 hours there appears a very pale white, almost colorless streak along the line of inoculation which has a glistening appearance. Isolated colonies are about 0.2 to 0.5 mm. in diameter, convex with granular edges. There is acid production, but this is not abundant. Lactose litmus-agar shows a red coloration along the streak on the second day. Only after about 4 days is there a reddening of the surrounding medium, and even then this is not very extensive. A peculiar penetrating odor arises from both tubes and plates.

Potato Cultures.—Upon this medium the growth is not very vigorous, but is present as a slight yellowish coloration along the line of inoculation after 4 days.

Milk.—A change is noted in the consistency of the cream after 4 days. Other than this no change is to be recognized in milk cultures kept in the incubator for 6 weeks.

Blood Serum.—Natural blood serum coagulated was used. The growth is very similar to that obtained upon agar, but is not nearly so vigorous. The growth along the line of inoculation is seen after a few days to broaden slowly if the tube be kept in the incubator.

Temperature Relationships.—The germ grows at ordinary temperature and also at that of the blood. It resists a heat of 59° C. for 10 minutes; it will not withstand one of 60° C. for that length of time. These relationships were determined according to the instructions laid down in Sternberg's Text-book of Bacteriology, 1896. As already pointed out by me the germ is evidently capable of resisting a temperature below 0° C. for several days and of —27° C. for several hours, although evidently this extreme cold led to some attenuation.

Action of Disinfectants.—A 1 per cent solution of carbolic acid destroyed the life of the germ in 5 minutes. A half per cent solution of hydrochloric acid was fatal in the same time, as was also a half per cent solution of sulphuric acid. The commercial hydrochloric and sulphuric acids were employed in these experiments.

RESULTS OF INOCULATION.

Poultry.—A fowl inoculated intravenously with 2 cc. of a culture 24 hours old was found dead on the second day. Further than this the virulence for fowls has not been determined, on account of an accident, owing to which the virulent germ was lost. A few hours after inoculation the fowls became listless. Even with the most attenuated virus a few hours after inoculation a profuse diarrhœa was noticed, the stools consisting of semi-mucoid masses of a greenish-yellow or white color. The autopsy showed as follows: Intestine filled with gas, crop empty, gizzard filled with small stones and ingesta. Intestine contained a greenish-yellow, semi-mucoid mass, kidneys normal, spleen congested and enlarged. Kidney fatty. Gall-bladder much distended. Heart and lungs normal. Where the fowls had been given a subcutaneous injection of a less virulent form into the breast muscle, at the point of injection was a mass of cartilaginous hardness, of a yellow color, free in the muscle tissue. The bacilli were found in all the fluids and tissues of the body.

Immunity has been produced in young chickens by the injection of a moderate quantity of an attenuated germ.

Turkey.—A turkey was inoculated intravenously with 6 cc. of a 24-hours-old culture and showed symptoms about an hour afterwards. At this time he was loath to stand; the feathers were slightly ruffled and the head drawn close to the body. The symptoms further than this

were not noted, as the animal was inoculated at night and found dead in the same spot on the following morning, having succumbed in about 15 hours. The autopsy, made a few hours after death, revealed the following: Liver, great congestion, otherwise normal. Heart, lungs, spleen and kidneys normal. Intestine, pale externally. From within, the mucosa was seen to be swollen and greatly congested, showing ecchymoses and areas of necrosis. This congestion was not localized, but extended from the gizzard to the anus. The intestine was filled with partially digested food of a greenish color. Crop greatly distended with ingesta. The mucous surfaces, from the mouth to the gizzard, were studded with ecchymoses and showed congested areas. This was also true of the mucous surfaces of the cavities of the head. The mucosa of the gizzard also showed congested areas.

*Rabbits.**—The first rabbit was inoculated through the digestive tract, and death occurred on the twelfth day. The disease followed the course of a septicæmia, the symptoms of which were characteristic. Another animal inoculated with a culture, 24 hours old, from this first died two days later. After this the germ rapidly gained virulence, the last rabbit, which was the ninth of the series, dying 6 hours from the time of inoculation. This last rabbit was inoculated with 3 cc. of a preparation of ten drops of blood from the previous animal to 10 cc. of broth. The lesions presented at the autopsy were as follows: Upon opening the abdominal cavity there was noted acute peritonitis with a slight effusion of serum. The intestine was distended with gas. The mucosa of the stomach peeled off with ease, the surface being studded with small ecchymoses and congested. The intestine was filled with a greenish-yellow, semi-mucoid mass. The mucosa was injected. Appendix discolored, mucosa congested with small ecchymoses. The large intestine contained gas and a dark semi-fluid mass. Liver firm, gall-bladder greatly distended. Kidneys slightly congested. Heart and lungs normal. The mucosa of the uterus showed great congestion and partial necrosis. The bacillus was found in the blood, and cultures from the various organs also revealed its presence. In this case it was demonstrated in the vitreous humor of the eye.

The symptoms of the inoculation disease in rabbits were marked. There was drowsiness. The hind legs were drawn well under the body.

* Evidently the conditions under which the bacilli were gained, namely, from the bodies of animals which had been dead for several days, and had been preserved at a temperature far below 0° Centigrade, had induced a very definite attenuation of the microbes.

The forelegs were drawn as far back as possible. In the disease produced by the virulent germ there was profuse diarrhœa beginning a few hours after inoculation, and in those withstanding for some time small tubercle-like masses were found in the appendix. Rabbits inoculated with the most virulent virus developed symptoms of an acute disease, remaining motionless until death supervened. In the disease produced by the attenuated germ the symptoms were similar, but the animal took food up to about 24 hours before death, at which time acute symptoms developed.

A guinea-pig inoculated with 1 cc. inside the thigh developed a local abscess.

Dogs fed on dead subjects developed no marked symptoms save a profuse diarrhœa. There was no elevation of the temperature.

The bacillus here described corresponds closely in all respects with the bacillus of European chicken cholera. So close is the resemblance that I cannot but hold that the two are identical, and that I have been dealing with an epidemic of the true disease, the first recorded in America. In only two respects have I found slight divergences from the statements of previous observers, namely, in the resistance of the organism to heat and disinfectants. The European germ is said by Baumgarten to be destroyed by an exposure to 55° C. for fifteen minutes or, according to Salmon, by an exposure to 56° C. for ten minutes. The form here described was killed only by a temperature of 59° C. with ten minutes' exposure. On the other hand it would appear to be more sensitive to disinfectants, for whereas Hueppe found that the European germ was killed by 3 per cent carbolic acid in six hours, the Canadian form did not grow after being left in 1 per cent carbolic acid for five minutes. The discrepancy in both cases would appear considerable, but it must be remembered that the want of agreement in the results of various observers in connection with these two tests of thermal death-point and disinfectant action are notorious, so that, when these two tests alone yield divergences, we do not possess sufficient grounds for stating that we are dealing with separate species.

From the form already recognized in America the separation is clearly marked. The Canadian germ is smaller, takes the polar stain

well, does not saponify milk, produces acid in dextrose and lactose broths, can be rendered most virulent for rabbits so that a drop or two of the blood of an affected rabbit will lead to the death of another in six hours, while both in fowls and rabbits there is developed a most profound diarrhœa. In addition to these differences I have found, in comparing the growths, that the American form flourishes much more vigorously in gelatine (stab and streak cultures), so that the growth is already recognizable in twenty-four hours, while the Canadian and European forms show nothing until the fourth day. On the other hand in plate cultures the colonies of the American form are much smaller, with more sharply-defined borders. There is little or no difference upon agar.

The study of the whole of the large group of bacilli of hæmorrhagic septicæmias has revealed so much variability, so many individual points of difference between microbes in the main closely resembling each other, that I shall not venture to say that the forms isolated by Dr. Moore belong to a distinct species. I only urge that the Canadian form approaches much more nearly to the classical type.

Before closing this section of my paper attention may be called to what, I believe, has not previously been noted, namely, that the turkey is very susceptible to inoculation chicken cholera.

UPON THE COMPARATIVE PRODUCTION OF ACID BY ALLIED BACTERIA AS A MEANS OF SPECIES DIFFERENTIATION.

In studying these three allied forms of bacteria I was led, thanks to Professor Adami, to pass beyond the limits of my initial research. His suggestion was that the comparative acid and alkali production by various species of bacteria had been insufficiently studied and that possibly a careful examination of such acid and alkali production might reveal a further means of differentiating allied forms. Acting upon this idea I have spent several months endeavoring to arrive at satisfactory conclusions with regard to the subject. Even now I cannot consider that more than preliminary studies have been made, nor should I publish at the present time were it not probable that an indefinite period may elapse before I can again take up this subject, while it is possible

that the record of my results obtained thus far may be of immediate use to others about to enter into like investigations.

In the first place I found that the fowl-cholera organisms resemble most of the pathogenic microbes in producing acid during the earlier stages of their growth.

To determine the amount of acid quantitatively the media had to be carefully prepared. In the earlier experiments meat infusion was employed, made strictly in accordance with the directions given by Abbott in his *Principles of Bacteriology*. This broth was neutralized with sodium hydrate, phenolphthalein being employed as an indicator. That no appreciable amount of muscle sugar was present in the broth so prepared was determined by the fermentation-tube test, the bacillus coli being employed as the testing germ.* Four tubes were then taken containing 10 ccm. of the neutral broth and were sterilized in the autoclave. Of these, two were inoculated with the germ; the other two were used as controls.

The following is an example of the result of titration at the end of 24 hours' growth at 37° C. The figures given indicate the number of cubic centimetres of a decinormal solution of sodium hydrate required to neutralize the broth, phenolphthalein being here, as elsewhere, employed as the indicator:

TABLE I.

Tube inoculated with Canadian chicken cholera.	No. 1	1.00
" " " " " "	" 2	1.10
Sterile tube, not inoculated.	" 3	0.15
" " " "	" 4	0.20

It will be seen that the process of sterilization of the broth in the tubes after the broth had been neutralized, and the subsequent keeping of these tubes at 37° C., had led to a slight acid production in the broth, but the amount was very small as compared with the acid production in the inoculated tubes, in each of which the amount of acid was almost the same.

I had prepared a large quantity of the broth made from meat infusion, and used in the above series of experiments, and part of it I next employed for a fuller experiment. Taking 20 tubes I placed in each, as before, 10 cubic centimetres of the neutral broth and then sterilized.

* Theobald Smith's ready method of making sugar-free bouillon (*Journal of Experimental Medicine*, ii (1897), 546) was published subsequent to the completion of this paper.

Three of the tubes were inoculated with a diplobacillus, sent to me by Dr. V. A. Moore and stated to have been isolated from diphtheritic lesions in fowls. Three were inoculated with the United States bacillus of fowl cholera, for which again I am indebted to Dr. Moore. Four were inoculated with the Canadian fowl cholera, four with anthrax, while five were not inoculated and were carried through as controls. In order that the amount of culture placed in each tube of the series might be the same, and might contain the same number of bacilli, I employed, not the platinum loop, but Pasteur pipettes, drawn out into fine capillary tubes. These were sterilized, and for each series one was filled with a 24-hour-old broth culture of one or other germ, and from it one drop was allowed to fall into each tube to be inoculated. Tubes so inoculated were placed in the incubator for 24 hours, at the end of which time they were titrated in the usual manner. Here again, in the table that follows, the figures given indicate the number of cubic centimetres of a decinormal solution required to neutralize the broth. It will be seen that the control tubes were in this case slightly alkaline and required an addition of acid for neutralization. Evidently the neutralization had originally been carried a little towards the alkaline side.

TABLE II.

Tubes.	1.	Uninoculated, employed as control	0.1
	2.	" " "	0.1
	3.	" " "	0.0
	4.	" " "	0.1
	5.	" " "	0.1
	6.	United States fowl diphtheria	0.3
	7.	" " " "	0.1
	8.	" " " "	0.05
	9.	United States fowl cholera	0.75
	10.	" " " "	0.4
	11.	" " " "	0.3
	12.	Canadian chicken cholera	0.5
	13.	" " "	0.5
	14.	" " "	0.6
	15.	" " "	0.6
	16.	Anthrax	0.2
	17.	"	0.15
	18.	"	0.2
	19.	"	0.2

It cannot be stated that the results of these experiments were entirely satisfactory. In two forms, namely, in the two cultures obtained from

Dr. Moore, there was a variation in the acid production, so wide, indeed, that I have been unable to account for it satisfactorily. The cultures in all cases were pure, and the only marked difference that I could make out between the various growths in these two series was that the test tubes, while containing each 10 cem., were of unequal diameters, so that in some cases a larger and in others a smaller surface was exposed to the atmosphere. Another possible cause for this irregularity may be the fact that whereas the chicken cholera and anthrax germs had been constantly transplanted, the other two had not, having been but recently transplanted from tubes of some little age sent from Washington.

Upon endeavoring to repeat these observations and to reproduce a broth identical with that employed in this last experiment I found that the amount of neutralization, the presence of muscle sugar in minute quantities in the different broths, and still other differences rendered it almost impossible for me to hope to gain any scientific precision in my results by following the lines employed up to this time. Hence it became necessary to endeavor to settle the question of acid production by different species and by allied forms of the same species by the employment of accurately compounded synthesized media.

In pursuing this work I have tried the formulæ of most of those who have already endeavored to form satisfactory synthesized culture media, and have further studied some fifty media made up in accordance with my own ideas. The work was very interesting, though at times very discouraging. It was my aim not so much to arrive at the formula of a medium specially suitable for the fowl cholera germ, but rather one which could be substituted for ordinary broth in ordinary work. There exist several media upon which other pathogenic germs, like those of anthrax and the bacillus pyocyaneus, will grow, but singularly few upon which the bacillus of chicken cholera will flourish. This difficulty in getting the bacillus of chicken cholera to grow in these media is wholly in harmony with the observations of Pasteur, who noted that it would not grow in various vegetable infusions. The great difficulty is in the addition of those elements or salts which are required in very minute quantities, as again in the proper addition of the calcium and magnesium salts. These latter are inclined to precipitate at an inopportune moment, a most undesirable feature. Not being able therefore to gain any satisfactory synthesized media, I was, for this work on chicken cholera, finally compelled to fall back again upon the ordinary broth. Liebig's extract of beef was now used in place of meat infusion, for I have found it less liable to contain fermentable matter. Four grammes of the ex-

tract were taken, dissolved in distilled water; 1 per cent of Witte's peptone and ½ per cent of rock salt were added. These ingredients were thoroughly dissolved, neutralized to phenolphthalein with a caustic potash solution and sterilized, the tubes filled and once more sterilized. In all cases the media were tested in the fermentation tube with the bacillus coli to determine the absence of fermentable matter. All the media used so far have given a negative reaction.

With this medium the first results obtained appeared to be hopeful and to give further proof of the close relationship between the European and Canadian forms. Thus I inoculated three tubes with equal quantities of a culture of the Canadian germ and three from a culture of the European form from the laboratory of Professor Kitt in Munich and obtained the following results:

TABLE III.

1.	Uninoculated tube as check	0.2
2.	" " "	0.2
3.	Canadian chicken cholera	0.4
4.	" " "	0.4
5.	" " "	0.3
6.	European fowl cholera from Munich	0.3
7.	" " " " "	0.3
8.	" " " " "	0.4

These results seemed to indicate a very close relationship between the two germs, which, it may be added, were possessed of very nearly the same virulence.

Similar results were obtained when I employed broth of the same composition containing 1 per cent glucose and 1 per cent lactose. The method of preparation was the same as in the previous experiments, but here the determination was made at the end of 48 hours, and a viginti-normal solution of sodium hydrate was employed in place of a decinormal, the titration being thereby accomplished with much greater ease and sureness.

TABLE IV.

	Plain Broth.	1% Glucose.	1% Lactose.
Tubes as check, uninoculated	0.5	0.6	0.5
	0.3	0.8	0.6
European germ (Kitt)	0.7	2.7	1.1
	0.7	2.4	1.0
Canadian germ	0.7	2.5	1.0
	0.6	2.7	1.1

These figures again favored the contention that the two forms are very closely related, the difference between the acid production in the different media being very slight. It is interesting to note the great increase in acid production brought about by the addition of the sugars.

But a more extensive and very carefully conducted series of observations showed that no absolute reliance can be placed upon this feature of acid production, so far as regards the micro-organism of chicken cholera.

The following table shows the results obtained by determining the acid production at the end of 24 hours in tubes of the same broth as that used in the previous series of observations, these tubes being inoculated with equal amounts of cultures of fowl cholera germs that had been subjected to different influences. A viginti-normal solution of sodium hydrate was used, and the figures represent in ccm. the amount added to neutralize 10 ccm. of broth culture.

TABLE V.

	Neutral Broth.	1% Lactose.	1% Glucose.
No. 1	0.3	0.8	0.7
	0.35	0.8	0.6
	0.3	0.9	0.7
No. 2	0.2	2.0	4.5
	0.3	2.2	4.1
	0.3	2.4	5.1
No. 3	0.3	2.0	3.9
	0.3	2.1	4.3
	0.3	2.2	4.2
No. 4	0.4	1.3	1.7
	0.4	1.2	1.3
	0.4	1.2	...
No. 5	0.4	1.2	1.5
	0.4	1.1	1.9
	0.4	...	1.5
Uninoculated tubes carried as check	0.05	0.6	0.7
	0.04	0.7	0.7
	0.04	...	...

No. 1. Bacilli of European fowl cholera passed through the fowl 10 weeks previously, and cultures since renewed every third day. This germ proved fatal to a rabbit in 6 hours, 3 cc. being inoculated intravenously.

No. 2. Bacilli from Canadian outbreak, from a broth culture taken direct from one of the St. Anne's fowls and preserved in a sealed tube for about 5 months.

No. 3. Bacilli from Canadian outbreak. Broth culture made from rabbit which had been infected through the digestive tract and had

succumbed in 36 hours. This culture had been kept in a sealed tube about 4½ months.

No. 4. Bacilli from Canadian outbreak passed through series of rabbits from rabbit which had been inoculated intravenously and had succumbed in 6 hours. This culture had been hermetically sealed for 4 months.

No. 5. Bacilli from Canadian outbreak passed through a turkey. Culture from liver renewed every third day for 3 months and 9 days.

It is to be observed from this table that there is little variation between the different series in neutral broth, but this is far from being the case in those containing sugar. A difference of 0.1 ccm. of a vigintinormal solution of the alkali added to 10 ccm. of the culture—that is, of 1 per cent in the amount of solution added—is inconsiderable, and in determinations of this nature, even when made with the greatest care, cannot be regarded as indicating any profound differences in acid production. But if this table be compared with Table 3 it will be seen that forms here giving very closely allied results to those with plain broth, namely, the European and Canadian forms, gave in other determinations wider variations, while with sugar-containing broths they gave results which were closely parallel. It is unsafe, therefore, to depend upon the apparently satisfactory results shown in this one table concerning the acid production by allied forms in neutral sugarless broth.

When we compare the results gained from lactose and glucose broths we find still greater discrepancies. So marked are they that it is immediately evident that the variations in virulence of at least one member of the group of the bacilli of hæmorrhagic septicæmia are accompanied by equally wide variations in acid production, and so far as this table may be depended upon it would seem to a certain extent that these variations are inverse, for the least virulent forms (Nos. 2 and 3) produced most acid in both lactose and glucose broths.

It is quite probable that more stable forms of bacteria may give more constant results; but it is with these unstable bacteria that we have the greatest difficulties, and are most often led to question whether we are dealing with distinct species, or merely with varieties of a common form, and so far as these observations extend it would seem evident that little or no dependence can be placed upon determinations of comparative acid or alkali production as helping to solve the problem of relationships.

CONCLUSIONS.

(*a*). The bacillus isolated by me from the outbreak of choleraic diarrhœa corresponds closely in all essential particulars with that of European chicken cholera.

(*b*). It differs, consequently, from that isolated from previous outbreaks of choleraic diarrhœa in the United States.

(*c*). A study of the acid production by this and allied bacilli would seem to show that the amount of acid produced by so variable a microbe varies greatly and is incapable of affording a further means of distinguishing between allied forms.

ADDENDUM.

ON SUNDRY SYNTHESIZED MEDIA.

In pursuing the subject of synthesized media the paper of J. J. McKenzie,* of Toronto, was taken as a working basis. The various media as given there were tried with very little success; from these others were composed, the best of which will be found below together with the results obtained. In all there were about fifty formulæ tried, including five or six taken from McKenzie's paper. In all cases the media were neutralized, phenolphthalein being used as indicator.

The first of this series was very simple, as follows:

Potassium phosphate	0.15
Ammonium chloride	0.80
Glycerine	5.00
Water	100.00

Six tubes were prepared and were inoculated with diphtheria, European fowl cholera, Canadian fowl cholera, pyocyaneus, Asiatic cholera, and the germ of the Pictou cattle disease. At the end of 24 hours the tubes inoculated with the germs of European fowl cholera and Asiatic cholera showed only slight growths, while the others showed nothing. At the end of 72 hours pyocyaneus appeared. None of the growths at this time were very vigorous. This same formula with the addition of 0.1 gramme of potassium sulphate showed in addition to the above at the end of 72 hours a growth in the tube inoculated with the germ of the Pictou cattle disease. The growths in this mixture were not vigorous. The next medium which proved itself worthy of passing notice was as follows:

Potassium phosphate	0.4
Potassium nitrate	0.8
Asparagin	0.8
Lactose	0.5
Glucose	0.5
Water	200.0

* *Journal of the American Public Health Association*, October, 1895.

This was inoculated with the same germs as the previous tubes, save that a putrefactive form was substituted for the Asiatic cholera. In 15 hours all show a slight growth, while that of pyocyaneus is quite vigorous. Diphtheria seems to grow fairly well after the first day and increases considerably in cloudiness.

Another medium which proved to be superior to the foregoing was as follows:

Ammonium succinate	0.8
Potassium phosphate	0.4
Potassium sulphate	0.2
Ammonium chloride	0.3
Asparagin	0.5
Lactose	0.5
Glucose	0.5
Water	200.0

Six tubes were taken and inoculated with the bacillus violaceus, pyocyaneus, coli, the germ of the Pictou cattle disease, European fowl cholera and that from the Canadian outbreak. At the end of 24 hours there is observed to be a very profuse growth in the case of the first three, being equal in all to that obtained ordinarily in broth, while that of bacillus coli is much better than the best growth obtainable in the same time in broth. The other three tubes remained sterile. After 48 hours pyocyaneus brought forth its characteristic color.

A still better formula was as follows:

Ammonium phosphate	0.5
Potassium nitrate	0.5
Potassium chloride	0.3
Ammonium sulphate	0.2
Asparagin	0.5
Lactose	0.5
Glucose	0.5
Water	200.0

This was inoculated with the same germs as the preceding, with a similar result excepting that violaceus was much more vigorous than in the preceding. Agar plates were made showing a good growth of the three forms above mentioned, as also of bacillus mesentericus, but there was not the character to the colonies such as is seen where broth is used.

I will mention but one more which I think is the best tried. The formula is as follows:

Ammonium phosphate	0.5
Potassium nitrate	0.5
Potassium chloride	0.3
Ammonium sulphate	0.2
Ammonium succinate	0.5
Lactose	0.5
Glucose	0.5
Water	200.0

This is practically the same as the one above save that instead of asparagin, ammonium succinate is used. Here bacillus violaceus, pyocyaneus and bacillus coli grew well. This medium was made up also with 7 per cent gelatine and gave similar results in examining water as did gelatine prepared with ordinary broth.

The following results were obtained with an agar preparation of this medium as compared with ordinary broth agar:

	Number of Colonies.	
	Broth.	Synthesized medium.
1.0 cc. water, 48 hours,	70	2
1.0 cc. water, 48 hours,	81	6
0.1 cc. water, 48 hours,	14	0
0.1 cc. water, 48 hours,	11	0
Pyocyaneus	1248	18,720
B. coli	156	260
Violaceus	No growth on either.	

The colonies were large, very distinct, and the edges were well defined. On the whole the colonies which appear are much larger than those seen on broth plates of the same age. There seems to be in this medium some element which is in such a form that the bacillus pyocyaneus has a special affinity for it and which seems to greatly increase its growth.

From the work which I have done in this line it does not seem probable that a generally serviceable medium of simple formula can be prepared, as many of the elements are required in very minute quantities. Magnesium and calcium are needed to insure success, but the great difficulty, as previous workers on this subject have noted, is to get them in such a form that they will not precipitate when subjected to the heat of the sterilizer.

DIFFUSE SARCOMA OF THE CEREBRAL AND SPINAL PIA MATER.

By GEORGE H. WEAVER, M. D.
(*From the Pathological Laboratory of Rush Medical College.*)

Tumors of the soft membranes of the brain and spinal cord, especially those of a diffuse character, are comparatively rare, and a careful study of each individual example is of distinct value. The following case was kindly offered me for study by Prof. Ludvig Hektoen, to whom I am indebted for much assistance in the preparation of my report.

Primary tumor of cerebrum, secondary diffuse sarcoma of the cerebral and spinal pia mater.—W. C., male, aged 11 years, entered the Presbyterian Hospital December 31, 1896, in the service of Dr. A. C. Cotton. Clinical diagnosis, tubercular meningitis.

Family history negative. No tubercular or specific history obtainable. Present illness began September, 1896, with convulsions, in which the whole body was rigid and the eyes fixed. Before the convulsions the patient was dizzy and vomited; after them he would sleep for a time, and upon waking would be delirious. At times he was confined to bed.

Examination on admission: Confined to bed, poorly nourished, skin warm and moist, countenance staring and flushed at times, pulse regular and rapid, Cheyne-Stokes respiration, bowels constipated, pain in head and neck; only partly conscious, moves but little, but there is no paralysis; eyes bulging, pupils widely dilated, sight much impaired, the right eye insensible to light, the left to sensation; nystagmus marked, double optic neuritis; temperature 98.8° F., pulse 100. Hyperæsthesia over entire body. Pain on percussion over the cranium. *Taches cérébrales.* From this period until death there were recurring convulsive paroxysms, sometimes several in a day, and again only at intervals of several days. There were, later, delirium and involuntary evacuations. Death took place April 13, 1897.

Autopsy by Dr. Hektoen.—Body small and extremely emaciated; head large; thyroid gland large. Larynx and trachea normal. Pleuræ smooth

and empty. Over the anterior surface of the right lower lobe is a circumscribed area in which the pleura contains numerous grayish-yellow, pinhead and larger, disc-like nodules. The peribronchial lymphatic glands contain tubercular areas. Lungs normal save for circumscribed areas of grayish-red consolidation in the lower lobes. Heart small; endo- and myocardium normal; pericardium smooth. Aorta normal. Spleen, kidneys, pancreas, liver, stomach, intestine and adrenals normal. Mucous membrane of urinary bladder red, rough and covered with shreddy material.

Upon opening the cranial cavity a large amount of very slightly turbid fluid runs out. Dura mater normal. The pia mater upon the optic commissure, the medulla and the upper part of the spinal cord is the seat of a reddish, indistinctly nodular thickening. Around the apex of the left frontal lobe is a similar area. The entire posterior surface of the spinal cord is the seat of marked thickening, so that the pia is reddish and somewhat lobulated, and the volume of the cord appears to be greater than normal. It fills the dura, and when the latter is cut masses are forced out through the opening. The ventricles of the brain are greatly dilated, containing a clear fluid.

Anatomical diagnosis.—Sarcoma of pia mater of brain and spinal cord. Hydrocephalus. Broncho-pneumonia. Acute cystitis. Chronic tuberculosis of the peribronchial lymph glands and pleuræ.

Description of the brain.—There are irregular areas of a gelatinous, opaque, whitish-gray substance in the pia mater, scattered over the anterior surface of the medulla and the pons and spreading out over the lower surface of the lobes of the cerebellum. The largest area upon the cerebellum is on the right lateral hemisphere, being 4.5 cm. from before backward and 2 cm. wide at the thickest part. This lies parallel to the fissure between the lateral and median lobes, extending outward from the fissure. This area is not elevated above the surface but appears to involve the underlying substance. From this area a thinner growth extends continuously over the lower surface of the median lobe, becoming less dense as it approaches the left lobe. Upon the left lobe, lying in a position symmetrical to that on the right, is an area of growth 1.5 cm. antero-posteriorly and 0.75 cm. transversely. This is slightly elevated above the surface and somewhat firmer than the corresponding area on the opposite side. Scattered over the under surface of both lateral cerebellar lobes and along the front of the medulla and pons are areas of growth varying in size from those which are very minute to those 0.5 cm. in diameter. The largest of these are elevated above the

surface and are firmer than the larger areas upon the cerebellum. They appear to bear no relation to the vessels in their distribution. On the upper surface of the optic commissure and optic nerves at their origin are globular masses of growth 0.5 cm. in diameter, which are partly continuous, thus forming an irregular, uninterrupted mass along the optic commissure and nerves. The apex of the left cerebral hemisphere is soft and somewhat collapsed. The surface for about 3.5 cm. from the apex, on all sides except below, presents the same sort of growth as that found on the cerebellum. It is, however, of a more pinkish-gray color. The tissue underlying this surface growth is very soft. A few small discrete masses of growth are seen at short distances from the larger area over the surface of the left cerebral hemisphere.

The brain was hardened in Müller's fluid containing 5 per cent of formalin. On cutting the left cerebral hemisphere into vertical sections, each 2 cm. thick, it is found that the centrum ovale of the left apex is replaced by a tumor extending antero-posteriorly 5 cm.; its diameter at a depth of 2 cm. is 5 cm., and at a depth of 4 cm. is 4 cm. The growth is extremely soft, and of a pinkish-gray color. In the sections the tumor mass tends to fall away from the surrounding tissue. The lateral ventricle is empty, very much dilated, its wall smooth, and the choroid plexus unaltered. Corresponding sections of the right hemisphere show the lateral ventricle to be similar to the left. This hemisphere is otherwise normal.

The fourth ventricle is filled to distension with a reddish and in parts grayish tumor mass. This is very soft, contains much blood and is continuous with the walls of the ventricle. It measures antero-posteriorly 4.5 cm., transversely 3.5 cm., and vertically 2.5 cm.

The spinal cord.—The cord, which was hardened in 5 per cent formalin, appears very much enlarged in the dorsal and lumbar regions. In the lower dorsal region it measures 1.75 cm. antero-posteriorly and 2.5 cm. transversely. The growth externally is slightly nodular and uneven, opaque and whitish-gray. In the upper dorsal region the blood-vessels are large and prominent. The growth extends over and between the nerves of the cauda equina. On section the cord itself is found to be of about normal size, the apparent enlargement being due to a growth of new tissue about it. The tumor growth is most abundant posteriorly, and is thickest in the lower dorsal and lumbar regions, where it is 0.75 cm. in thickness; it becomes much thinner in the cervical and upper dorsal regions. The growth in front of the cord also extends throughout the entire length, being more largely developed in the lower dorsal

and lumbar regions. It is nowhere more than 1 or 2 mm. thick. Laterally the growth is more abundant in the lower portion of the cord, where it is from 2 to 3 mm. thick and completely surrounds the posterior roots and to a less extent the anterior roots of the spinal nerves. The posterior roots appear very large from the tumor deposit. In the upper part of the cord the lateral growth exists as a very delicate, thin layer.

MICROSCOPIC EXAMINATION.

Spinal cord.—Weigert's stain. The cord is usually normal except in some places along the posterior border, where it is invaded for a short distance by the tumor.

Hæmatoxylin and eosin. The tumor is usually sharply limited at the border of the cord by a fibrous structure consisting of the altered pia mater. There is a growth of tumor tissue along the normal trabeculæ which pass from the pia mater into the cord. Posteriorly the cord is directly invaded by the tumor for a short distance. The tumor is everywhere of the same structure, consisting of closely-packed, small, round cells, with deeply-staining nuclei. The intercellular structure is usually but little developed, and in most places appears as a homogeneous material, staining faintly with eosin. Where the cells are not so closely packed it is finely fibrillated. In some places the connective tissue framework is more largely developed, appearing as quite heavy bands, but with little tendency to any regularity of arrangement. The blood-vessels are large and numerous with well-developed walls. In the cervical and upper dorsal regions the tumor is less than in the lower parts, but exists upon all sides. It is here thinnest at the sides and most marked posteriorly. The nerve-roots are here more or less surrounded by the growth, which has not penetrated the nerves themselves. In the lower dorsal and lumbar regions the growth is also least laterally and most developed posteriorly. The posterior nerve-roots, and to a less degree the anterior roots, exhibit an infiltration of the epineurium and perineurium, but the endoneurium has not been invaded by the tumor. Some of the larger vessels and bands of fibrous tissue have undergone hyaline degeneration. In the cauda equina the growth surrounds the nerves, but only in a few places was the endoneurium penetrated by tumor cells.

Brain.—The tumor in the apex of the frontal lobe consists of small round cells with deeply-staining nuclei, like those in the spinal growth. The arrangement of the vessels and the trabeculæ of the fibrous framework produces a somewhat alveolar appearance in places. The growth in the cerebral pia mater is similar to that in the spinal.

Sections were made of the tumor growth in the fourth ventricle, including the adjacent cerebellar tissue with its covering of pia mater, which appeared very slightly thickened. There is everywhere a thin layer of new growth following the pia mater over the convolutions of the cerebellum. The cells penetrate to a very slight extent the brain substance. The blood-vessels are very numerous, many being of large size. There are large and small areas of hæmorrhage into the tumor, some being quite recent with well-preserved blood corpuscles; others old, with remnants of blood-cells, fibrin and blood pigment.

Lungs.—In the lungs, on and near the pleura, are small areas of tubercular infiltration, containing foci of caseation, giant cells and tubercle bacilli. In the lungs are some areas of cell accumulation which resemble those in the tumor of the brain and cord. In these no caseation or giant cells are seen. From the histology of these areas it is impossible to decide whether they are metastatic tumors or not.

I have collected from the literature ten reports of diffuse meningeal sarcoma, of which the following are abstracts:

I. (Coupland and Pasteur.*) Female, aged 22 years. Course of illness about 4 months. Began with headache and morning sickness; later there were pains in the right shoulder and arm. A month before death pain along the spine; two days before death constant occipital pain radiating to the right shoulder and arm, double optic neuritis, paralysis of both external recti, tenderness of scalp, hyperæsthesia along the spine, weakening but not paralysis of the legs, and absence of knee-jerk; sphincters acted normally; no convulsions.

Autopsy.—Soft, jelly-like masses of new growth scattered over the meninges beneath the dura in the posterior fossa, about the body of the sphenoid and backward almost continuously to the foramen magnum. Medulla and pons uniformly invested with similar growth in which both sixth nerves were completely embedded. Spinal cord uniformly ensheathed in a semi-translucent, softish growth of a grayish-white color, the tumor being most developed on the posterior and lateral aspects of cord. Nerves of cauda equina surrounded by thin sheaths of new growth, which at intervals expanded into small tumors. The cord itself normal in shape and appearance throughout. The new growth was a small round-celled sarcoma, which followed the trabecular ingrowths of the pia into the cord. Brain normal.

* *Trans. Path. Soc.*, London, xxxviii (1887), 26.

II. (Coupland and Pasteur.*) Female, aged 4½ years. Duration of illness about 3 months. Following a fall the child became irritable, developed a squint, and at the end of a month was unable to walk. Pain in upper part of back and head was followed by sudden blindness six weeks from the onset. Two months from the beginning was conscious and intelligent; pupils dilated and insensitive, blindness, pain in back and head. Optic discs gray and indistinct. General muscular weakness without atrophy, normal tactile sense, *taches cérébrales*. No convulsions.

Autopsy.—A layer of grayish material covered the under surface of the cerebellum over the middle and part of the lateral lobes, varying in thickness and extending for about half an inch into the nervous tissue. Middle lobe almost entirely replaced by a soft, gray, vascular growth. Pia-arachnoid over medulla and pons normal. Thin layer of new growth on left side of optic chiasm and nerve, with similar patch, about the size of a pea, on under surface of left lower temporal convolution. Lateral ventricles greatly distended. Basal ganglia normal. Spinal cord appeared enlarged at upper part with small scattered hæmorrhages in the gray matter. Posterior aspect of the cord covered throughout by new growth ¼ to ⅓ inch thick in lumbar region. The tumor was a small round-celled sarcoma.

III. (Ollivier.†) Male, aged 11 years. Duration of disease about 6 months. There were paroxysmal headaches, retraction of head, convulsions, and later vomiting. Two days before death blindness with dilated pupils and pains in the limbs. The day before death acute pains throughout the body, but worst in the back; sensation and motion unimpaired. Patient conscious. Convulsions, constipation and nausea; no vomiting.

Autopsy.—There was a uniform pinkish formation extending over the posterior surface of the cord, averaging one line in thickness, thinner toward lateral aspects of the cord and blended with arachnoid. It was fairly firm, like unsoftened cerebral tissue, and traversed by small blood-vessels. There was a tumor in the cerebellum.

IV. (Hadden.‡) Although described as chronic cerebro-spinal meningitis, this case was evidently one of diffuse meningeal sarcoma. Male, aged 13 years. Duration 14 to 17 months. An injury to the head 17 months before death was followed by pain in the head and vom-

* *Trans. Path. Soc.*, London, xxxviii (1887), 26.

† Traité des maladies de la moelle épinière, t. ii, p. 490, obs. cxxxviii, Paris, 1837.

‡ *Trans. Path. Soc.*, London, xxxvi (1885), 13.

iting. 14 months before death sickness in the morning, pain in the head, fainting and attacks of dizziness. Two or three months before death weakness of legs, loss of control over bladder, convulsions, followed by stupor, occurring as frequently as 4 or 5 times a day and ceasing a few weeks before the fatal ending. The patient was drowsy and nearly blind, the pupils being equal, dilated and sluggish. Temperature usually normal or sub-normal, being only twice above 100° F.

Autopsy.—Under surface of cerebellum almost entirely coated with an opaque white material varying in thickness from 1 to 4 mm. Upper surface of cerebellum and adjacent parts of the cerebrum similarly but less extensively affected. Tips of temporosphenoidal lobes showed the same condition in a minor degree. Much thickening and white opacity about optic tracts and chiasm. Lateral ventricles distended with clear fluid. No tumor in the brain. Beneath arachnoid on posterior surface of lower dorsal and lumbar cord a thick, white layer similar to that upon cerebellum, which presented numerous bleb-like elevations. Cervical and upper dorsal cord free.

The white thickening over cerebellum and cord consisted microscopically of round and spindle-shaped cells with a considerable amount of new connective tissue in interlacing bundles. The vessels were large. No tubercles.

V. (E. L. Fox.*) Male, aged 29 years. Duration of disease not given (patient came to hospital after being sick for three months, and probably did not live long afterward); began with shooting pains in arms. Gradual flexion of fingers developed. Difficulty in urinating and priapism. On admission anæsthesia of legs, thighs, abdomen and chest to third rib, and paralysis of lower extremities.

Autopsy.—The cord seemed generally larger than normal with a marked swelling posteriorly at its upper portion midway between cervical bulb and second cervical vertebra. On section this was found to be a tumor, 1½ in. long and ¾ in. broad, with well-defined margins of a purplish color and tolerably firm. Cord smaller than normal, the great apparent increase in size being due to a layer of yellowish-gray substance fully ¼ in. thick, enveloping cord on all sides. This growth extended from highest portion of cord to cauda equina. In pia mater covering pons and medulla much thickening and rounded masses of exudate extending along the vessels as far as fissure of Sylvius.

Microscopically the tumor was a spindle-celled sarcoma.

* *Bristol Medico-Chirurg. Journ.*, i (1883), 104.

VI. (G. H. Bennett.*) Male, aged 48 years. Duration about 11½ months. Began with weakness in legs. No headache or other cerebral symptoms except dizziness. Vision good. Dull aching in back and lower limbs. For six weeks before death patient could not stand; suffered from constant severe pains in legs and from attacks of uncontrollable vomiting.

Autopsy.—Areas of softening in cerebrum and cerebellum. Pia mater of cord thickened, especially its posterior aspect, most marked below and gradually diminishing upward. Posteriorly, membrane studded throughout with round and oval, isolated and confluent, white nodules, varying in size from mustard seed to pea. These growths surrounded posterior roots. None visible to naked eye in front or laterally. The growth was a round-celled sarcoma.

VII. (Westphal.†) Female, aged 15 years. Duration a little over a month. At first headache, dizziness and pain in back, and two weeks later convulsions. Mind dull, pupils irregular, slight paresis, pain over spine on percussion, no choked disk. Before death severe headache, dizziness, vomiting, paralysis of ocular muscles and absence of knee-jerk. No fever. Death took place in a convulsion.

Autopsy.—Grayish-white and in parts more reddish thickening of pia-arachnoid, extending from optic chiasm backward over pons and medulla and outward somewhat over temporal lobes. Cranial nerves more or less imbedded in tumor thickening. Lateral ventricles contained blood-tinged fluid. In and near left optic thalamus three firm, smooth tumors of a red and grayish-white color, one being connected with tela choroidea. Pia-arachnoid of cord, especially posteriorly, presented grayish-red tumor growths, slight above, but increasing downward, becoming in dorsal region a prominent covering. Cord itself normal. Metastatic nodules in lung.

The tumor was a richly nuclear, round-celled sarcoma, with numerous vessels scattered between the cells. The cord was not invaded. The spinal nerve-roots were imbedded in but not invaded by the growth. The tumor had implicated the cranial nerves.

VIII. (Virchow.‡) Male, aged 30, copper-worker; attacks of colic, paresis and toward the last almost total blindness.

Autopsy.—On surface of hemisphere single yellowish-brown pigment spots. At base of brain a more diffuse, confluent deposit in the soft membranes of a melanotic material, extending laterally and thickest be-

* *Trans. Clin. Soc.*, London, xviii (1885), 168.
† *Arch. f. Psychiatrie*, xxvi (1894), 770.
‡ Virchow's *Archiv*, xvi (1859), 180.

tween chiasm and pons. At apex of middle lobe and on sides and middle of cerebellum growths as much as 3 lines in thickness. Diffuse growth along various cranial nerves, optic and olfactory being imbedded in it. The growth extended into the nerves and completely infiltrated the Gasserian ganglion, but did not extend beyond the foramina. Eyes normal. On posterior surface of cord in upper part isolated, flat, almost black tumors; below in dorsal and lumbar regions these became continuous, the cord being covered by a layer of growth 2 to 3 lines thick. In cervical portion, posteriorly and laterally and in front throughout, only blackish-brown pigmentation was apparent. Nerves of cauda equina and most of other spinal nerves covered by growth forming in places nodules. The tumor was a round and spindle-celled melanotic sarcoma. In a few places pigment was absent.

IX. (G. Lentz.*) Male, aged 23 years. Duration about 12 months. Began with pain in back, later in arms and legs; weakness of muscles, first in legs, later in arms and trunk. At end of 6 months pain and tenderness on percussion over spine and exaggerated patellar reflexes. Optic neuritis with normal pupillary reaction. One month before death patient was bed-ridden, mind dull, pupils irregular and sluggish, bilateral choked disk, marked paresis and atrophy of muscles. Patellar reflex present on right side only. Sensibility reduced in legs, arms and parts of back. Later vomiting and cystitis.

Autopsy.—There was a tumor involving corpus callosum and laterally invading the hemisphere. Lateral ventricles distended with fluid. A cavity in upper part of cord. Whole posterior extent of cord covered by a pale gray mass about 1 mm. thick. In dorsal region growth extended to the sides of cord. From lumbar region downward whole cord encased in a gray, homogeneous, jelly-like mass 0.5 cm. thick. Cord was of normal appearance only in the lower portion.

The mass of the tumor consisted of closely-packed, short, oval or roundish cells, separated by scanty interstitial tissue, in places distinctly fibrillated. There were abundant vessels with well-developed walls, some having undergone hyaline changes. The pia was usually sharply differentiated from the cord, and in places the tumor penetrated along the pial septa. Here and there the connective tissue was arranged so as to form a meshwork in the spaces of which the cells were accumulated. Large cells of endothelial type were found singly and at times in groups. Limited portions of cord were directly invaded by the tumor. The cerebral tumor was of similar structure and hæmorrhagic.

* Ziegler's *Beiträge*, xix (1896), 663.

X. (R. Schulz.*) Female, aged 16 years. Duration about 3 months. Severe pain in back, numbness and formication in hands and arms, with increasing weakness in arms, which were paralyzed shortly before death. Patient conscious up to the last. Pain in head, nutrition good, inability to stand, hands red and swollen, tenderness on pressure over main nerves of trunk and upper limbs. No affection of cerebral nerves. Marked tenderness over whole spine, retention of urine, constipation; anæsthesia of both upper limbs and absence of the patellar reflex on right side. Clinical diagnosis was acute meningo-myelitis.

Autopsy.—Cranium and its contents normal. On opening spinal dura cord bulged, presenting a sausage-like appearance. Sub-arachnoid space occupied throughout by grayish-red mass of growth, covered by delicate arachnoid, nodular and lobulated, partly firm, in places soft and studded with hæmorrhages. In lumbar and lower dorsal regions growth completely ensheathed cord, while in the upper part it covered only posterior surface. At one point it penetrated the cord.

Microscopically growth was made up of large endothelial cells, traversed by numerous capillaries which gave it an alveolar character. It is described as an alveolar sarcoma.

Of the 11 cases, including the one here reported, in which there was a diffuse sarcomatous growth in the pia mater of the cord and base of the brain, in 6 there was no tumor of the nervous structures which could be considered primary (Coupland and Pasteur, Schulz, Hadden, Fox, Virchow). In the other five there was tumor growth in either the cerebrum, cerebellum, pons or basal ganglia. In all of these cases the macroscopic characteristics were very similar, as was also the distribution. In the cranial pia mater the growth was most abundant about the optic commissure and over the cerebellum, and spread here and there to the surface of the lower portions of the cerebrum. It usually extended more or less along the cranial nerves. The spinal growth was most extensive along the posterior surface of the lower part of the cord. The posterior growth was comparatively slight in the upper part, as was also that in front and to the sides of the cord throughout the entire length. The tumor tissue was yellowish or grayish-white, with more or less of a pink or red color in some cases. The consistence varied, but was usually rather soft.

* *Archiv f. Psych.*, xvi (1885), 592. (Abs. in *Trans. Path. Soc.*, London, xxxviii (1887), 32.)

The microscopic characters of the tumors from the various cases have differed somewhat. Virchow's case is the only one of a melanotic growth. Schulz reports his case as one of alveolar sarcoma. In Hadden's case the tumor is said to consist of round and spindle cells, and Fox's was one of spindle-cell growth. In the two cases of Coupland and Pasteur, in those of Bennett, Lentz and Westphal, and in my case, the tumor was a small, round-celled sarcoma. In some of the cases the blood-vessels are spoken of as being large and abundant. There was usually little or no tendency for the growth to invade contiguous structures. The cord was rarely involved, and then only to a slight degree; the brain was more apt to suffer. The growth, however, showed a special tendency to infiltrate the pia mater even to its finest processes and prolongations. The cranial nerves were occasionally invaded by the tumor cells, but the spinal nerve-roots, even when imbedded in a mass of tumor tissue, were only occasionally directly attacked.

The variety of diagnoses made *intra vitam* in the reported cases indicates the divergent character of the clinical manifestations. This is largely due to the wide diffusion of the lesions and the association in many of the cases of symptoms produced by one or more tumor nodules in the brain. In my case the large growths in the cerebrum and fourth ventricle had not given rise to symptoms, and it was thought by the clinicians who had studied the case that localized tumor could be excluded from the diagnosis.

The ages of the patients varied from 4½ to 48 years. Of those in which the age is recorded, 6 were under 20 years and only one above 30 years. The course was usually tolerably rapid, the fatal termination being reached within a few months. Pain in the head and back was an early symptom. There were often pain and hyperæsthesia in the extremities, followed by muscular weakness, and later paralysis. Constipation was sometimes followed by incontinence of fæces and urine. Paralysis of the ocular muscles, partial or complete loss of sight and optic neuritis were noted. The temperature was not usually above the normal. In the early stages consciousness was often unimpaired, but later was sometimes much disturbed.

INDEX OF VOL. III

PAGE

Abdominal Cavity, Teratoma of (Montgomery) 259; — (Barker.) 284

Acid Production by Bacteria (Higgins) 651

Adipose Tissue, Action of Pancreatic Tissue on (Williams) 585

Albuminuria, Physiological (Sollmann & McComb)..... 137

Anatomy, Application of Roentgen Rays to Study of (Codman) 383

Aneurism, Aortic, Tufnell and Calcium Treatment of (Taylor) 371

Antitoxin, Relation of the Toxicity of Diphtheria Toxin to its Neutralizing Value (Park and Atkinson) 513

Aortic Aneurism, Tufnell and Calcium Treatment of (Taylor) 371

ATKINSON, J. P. See Park, W. H.

Bacilli of Bovine and Human Tuberculosis (Smith) 451;—Bacillus Pyocyaneus in Dysentery (Lartigau) 595;—Bacillus Tuberculosis in Sputum (Craig) 363;—Bacillus of Typhoid in Urine (Richardson) 349

Bacteria, Comparative Production of Acid by Allied Bacteria (Higgins) 651;—Bacteria, Importance of Lesion in Animal Tissue for Lodgment and Multiplication of (Cheesman & Meltzer) 533;—Bacteria, Inoculation of, into Spleen of Living Animals (Cheesman & Meltzer) 533

PAGE

BARKER, L. F. Note on Dr. Montgomery's Case of Teratoma 284

Birds, Hæmocytozoa of (Opie) 79;—(MacCallum) 103, 117

Blastomycees in a Case of Pseudo-Lupus Vulgaris (Gilchrist & Stokes) 53

Blood, Effect of Tufnell Method on, etc. (Taylor)..... 371

Bovine Tubercle Bacilli (Smith) 451

Branched Form of Bacillus Tuberculosis in Sputum (Craig) 363

BROWN, T. R. Trichinosis, with Especial Reference to the Increase of the Eosinophilic Cells in the Blood and Muscle, the Origin of these Cells and their Diagnostic Importance 315

Calcium Salts, Effects in Treatment of Aneurism (Taylor). 371

CHEESMAN, T. M., and MELTZER, S. J. Direct Inoculation of Bacteria into the Spleen of Living Animals; Importance of a Lesion in Animal Tissue for the Lodgment and Multiplication of Bacteria..... 533

CODMAN, E. A. Application of the Roentgen Rays to the Study of Anatomy..... 383

COUNCILMAN, W. T. Acute Interstitial Nephritis 393

CRAIG, C. F. Branched Form of the Bacillus Tuberculosis in Sputum 363

CUNNINGHAM, R. H. Experimental Thyroidism 147

PAGE

Degeneration of Striated Muscle in the Uvula (Hoen)..... 549
Diphtheria Toxin (Park & Atkinson) 513
Discontinuous Sterilization, when Ineffective (Smith).... 647
Dysentery, caused by Bacillus Pyocyaneus (Lartigau) 595

Eosinophilic Cells in Trichinosis (Brown) 315
Exudates, Fibrinous (Gaylord) 1

Fat Necrosis (Williams)..... 585
Fat, Serous Atrophy of (Herter) 293
Fat Starvation with reference to the Production of Serous Atrophy of Fat (Herter).... 293
Fibrinous Exudates and Fibrinoid Degeneration (Gaylord) 1
FLEXNER, S. Pseudo-Tuberculosis Hominis Streptothricha 435
Fowl Cholera (Higgins)..... 651

GAYLORD, H. R. Fibrinous Exudates and Fibrinoid Degeneration 1
Giant Cells in Absorption of Coagulated Blood Serum in Rabbit's Eye (Hektoen)..... 573
Giant Cells in Healing Tuberculous Tissue (Hektoen).... 21
GILCHRIST, T. C., and STOKES, W. R. Pseudo-Lupus Vulgaris caused by Blastomyces 53

Hæmocytozoa of Birds (Opie) 79;—(MacCallum) 103, 117
HEKTOEN, L. Fate of the Giant Cells in the Absorption of Coagulated Blood Serum in the Anterior Chamber of the Rabbit's Eye, 573;—Fate of Giant Cells in Healing Tuberculous Tissue, etc..... 21
HERTER, C. A. Fat Starvation with especial reference to the Production of Serous Atrophy of Fat 293

PAGE

HIBBARD, C. M., and WHITE, F. W. Leucocytosis of Labor and the Puerperium..... 639
HIGGINS, C. H. Epidemic of Fowl Cholera and the Comparative Production of Acid by Allied Bacteria 651
HOEN, A. G. On a Form of Degeneration of Striated Muscle in the Uvula..... 549
HOWELL, W. H. Physiological Effects of Extracts of the Hypophysis Cerebri and Infundibular Body 245
Hypophysis Cerebri, Physiological Effects of Extracts of (Howell) 245

Infundibular Body, Physiological Effect of Extracts of (Howell) 245
Interstitial Nephritis (Councilman) 393

Labor, Leucocytosis of (Hibbard & White) 639
LARTIGAU, A. J. Pathogenesis of Bacillus Pyocyaneus.. 595
Leucocytosis of Labor and the Puerperium (Hibbard and White) 639
Lupus (Pseudo) Vulgaris (Gilchrist & Stokes) 53

MACCALLUM, W. G. Hæmatozoan Infection of Birds, 117;—Pathological Changes in the Organs of Birds Infected with Hæmocytozoa 103
MCCOMB, E. C. See Sollmann, T.

Madura Foot (Wright)..... 421
MALLORY, F. B. Histological Study of Typhoid Fever..... 611
MELTZER, S. J. See Cheesman, T. M.
MONTGOMERY, D. W. Teratoma of the Abdominal Cavity..... 259
Muscle, Degeneration of, in Uvula (Hoen) 549
Mycetoma (Wright) 421

PAGE

Nephritis, Acute Interstitial (Councilman) 393
Nerve Fibres, Regeneration of, in Central Nervous System (Worcester) 579

OPIE, E. L. Hæmocytozoa of Birds 79

PARK, W. H., and ATKINSON, J. P. Relation of the Toxicity of Diphtheria Toxin to its Neutralizing Value upon Antitoxin in Different Stages in the Growth of Culture.... 513
Pancreatic Tissue, Action on Subcutaneous Adipose Tissue (Williams) 585
Pathogenesis of Bacillus Pyocyaneus (Lartigau) 595
Physiological Effects of Extracts of the Hypophysis Cerebri and Infundibular Body (Howell) 245
Pia Mater, Diffuse Sarcoma of (Weaver) 669
Pseudo-Lupus Vulgaris caused by a Blastomyces (Gilchrist & Stokes) 53
Pseudo-Tuberculosis Hominis Streptothricha (Flexner) ... 435
Puerperium, Leucocytosis of Labor and (Hibbard & White) 639
Pyocyaneus Dysentery (Lartigau) 595

Regeneration of Nerve Fibres in Central Nervous System (Worcester) 579
RICHARDSON, M. W. Typhoid Bacillus in the Urine..... 349
Roentgen Rays, Application to Study of Anatomy (Codman) 383

Sarcoma of Pia Mater (Weaver) 669
Serous Atrophy of Fat and Fat Starvation (Herter) 293

PAGE

SMITH, T. Bovine Tubercle Bacilli and Human Bacilli from Sputum, 451;—One of the Conditions under which Discontinuous Sterilization may be Ineffective 647
SOLLMANN, T., and MCCOMB, E. C. Physiological Albuminuria 137

Spleen, Inoculation of Bacteria into (Cheesman & Meltzer).. 533
Sputum, Bacillus Tuberculosis in (Craig) 363
Sterilization, Discontinuous (Smith) 647
STOKES, W. R. See Gilchrist, T. C.
Streptothrix in Pseudo-Tuberculosis (Flexner) 435

TAYLOR, A. E. Effects upon the Blood of the Tufnell Method and the Calcium Salts in the Treatment of Aortic Aneurism 371
Teratoma of the Abdominal Cavity (Montgomery), 259;—(Barker) 284
Thyroidism, Experimental (Cunningham) 147
Toxin of Diphtheria, Relation of its Toxicity to its Neutralizing Value upon Antitoxin, etc. (Park & Atkinson)..... 513
Tufnell Method, Effect upon the Blood, etc. (Taylor)..... 371
Trichinosis, with reference to Eosinophilic Cells (Brown).. 315
Tubercle Bacilli, Bovine and Human (Smith), 451;—Tubercle Bacillus in Sputum (Craig), 363;—Tuberculous Meningitis, Fate of Giant Cells in (Hektoen), 21;—Tuberculosis (Pseudo) Hominis Streptothricha (Flexner), 435;—Tuberculous Tissue, Fate of Giant Cells in Healing (Hektoen) 21

PAGE

Typhoid Bacillus in Urine (Richardson), 349;—Typhoid Fever, Histological Study of (Mallory) 611

Urine, Typhoid Bacillus in (Richardson) 349

Uvula, Degeneration of Striated Muscle in (Hoen)..... 549

WEAVER, G. H. Diffuse Sarcoma of the Cerebral and Spinal Pia Mater..... 669

PAGE

WHITE, F. W. See Hibbard, C. M.

WILLIAMS, H. U. Experimental Fat Necrosis: The Action of Pancreatic Tissue upon Subcutaneous Adipose Tissue... 585

WORCESTER, W. L. Regeneration of Nerve Fibres in the Central Nervous System..... 579

WRIGHT, J. H. Mycetoma (Madura Foot)..... 421

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

EDITED BY

WILLIAM H. WELCH, M. D.

Baltimore

ASSOCIATE EDITORS

FOR PHYSIOLOGY

H. P. Bowditch, M. D., Boston
R. H. Chittenden, Ph. D., New Haven
W. H. Howell, M. D., Ph. D., Baltimore

FOR PATHOLOGY

J. George Adami, M. D., Montreal
W. T. Councilman, M. D., Boston
T. Mitchell Prudden, M. D., New York

FOR PHARMACOLOGY

John J. Abel, M. D., Baltimore
Arthur R. Cushny, M. D., Ann Arbor
H. C. Wood, M. D., Philadelphia

FOR MEDICINE

R. H. Fitz, M. D., Boston
Wm. Osler, M. D., F. R. C. P., Baltimore
William Pepper, M. D., Philadelphia

VOLUME THIRD

No. 6.

NEW YORK
D. APPLETON AND COMPANY
LONDON: 33 BEDFORD STREET, COVENT GARDEN
GUSTAV FOCK - - - NEUMARKT, 40, LEIPSIC
MASSON ET CIE. - 120 BOULEVARD SAINT-GERMAIN, PARIS

November, 1898

Copyright, 1898, by D. APPLETON AND COMPANY
Entered at the Post Office at New York and admitted for transmission through the mails at second class rates

COLLABORATORS

A. C. ABBOTT, M. D., Professor of Hygiene, University of Pennsylvania.

L. F. BARKER, M. D., Associate Professor of Anatomy, Johns Hopkins University; Assistant Pathologist, Johns Hopkins Hospital.

JOHN S. BILLINGS, M. D., U. S. Army (retired), New York City.

HERMANN M. BIGGS, M. D., Professor of Materia Medica, Therapeutics, and Clinical Medicine, The University and Bellevue Hospital Medical College; Pathologist and Director of the Research Laboratory of the Health Department of New York City.

B. MEADE BOLTON, M. D., Director of the Laboratory of Hygiene, Board of Health of the State of New Jersey, Princeton.

T. M. CHEESMAN, M. D., Instructor in Bacteriology, Columbia University.

JOHN G. CURTIS, M. D., Professor of Physiology, Columbia University.

FRANCIS DELAFIELD, M. D., Professor of the Practice of Medicine, Columbia University.

GEORGE DOCK, M. D., Professor of the Theory and Practice of Medicine, University of Michigan.

H. H. DONALDSON, Ph. D., Professor of Neurology, University of Chicago.

E. K. DUNHAM, M. D., Professor of Pathological Anatomy, Bacteriology, and Hygiene, The University and Bellevue Hospital Medical College.

H. C. ERNST, M. D., Professor of Bacteriology, Harvard University.

SIMON FLEXNER, M. D., Professor of Pathological Anatomy, Johns Hopkins University.

GEORGE W. FULLER, B. S., Chief Chemist and Bacteriologist to the Commissioners of Water Works, Cincinnati.

J. GUITÉRAS, M. D., Professor of General Pathology and Morbid Anatomy, University of Pennsylvania.

WINFIELD S. HALL, Ph. D., M. D., Professor of Physiology, Northwestern University Medical School, Chicago.

H. A. HARE, M. D., Professor of Materia Medica and Therapeutics, Jefferson Medical College.

LUDVIG HEKTOEN, M. D., Professor of Morbid Anatomy, Rush Medical College, Chicago.

CHRISTIAN A. HERTER, M. D., New York City.

EUGENE HODENPYL, M. D., Assistant in Pathology, Columbia University.

C. F. HODGE, Ph. D., Assistant Professor of Physiology and Neurology, Clark University.

W. T. HOWARD, Jr., M. D., Professor of Pathology, Western Reserve University.

G. CARL HUBER, M. D., Assistant Professor of Histology and Embryology, University of Michigan.

WYATT JOHNSTON, M. D., Bacteriologist to the Board of Health, Province of Quebec.

E. O. JORDAN, Ph. D., Assistant Professor of Bacteriology, University of Chicago.

J. J. KINYOUN, M. D., Director of the Bacteriological Laboratory of the Marine-Hospital Service, Washington.

HENRY KOPLIK, M. D., New York City.

FREDERIC S. LEE, Ph. D., Adjunct Professor and Demonstrator of Physiology, Columbia University.

JACQUES LOEB, M. D., Associate Professor of Physiology and Experimental Biology, University of Chicago.

(*Continued on third page of cover*)

COLLABORATORS—(*Continued*)

W. P. LOMBARD, M. D., Professor of Physiology and Histology, University of Michigan.

GRAHAM LUSK, Ph. D., Professor of Physiology, The University and Bellevue Hospital Medical College.

A. B. MACALLUM, M. D., Professor of Physiology, University of Toronto.

J. J. MACKENZIE, B. A., Bacteriologist of the Provincial Board of Health of Ontario.

F. P. MALL, M. D., Professor of Anatomy, Johns Hopkins University.

F. B. MALLORY, M. D., Assistant Professor of Pathology, Harvard University.

S. J. MELTZER, M. D., New York City.

S. WEIR MITCHELL, M. D., Philadelphia.

DOUGLASS W. MONTGOMERY, M. D., Professor of Diseases of the Skin, University of California.

V. A. MOORE, M. D., Professor of Comparative Pathology and Bacteriology, Cornell University.

F. G. NOVY, M. D., Junior Professor of Hygiene and Physiological Chemistry, University of Michigan.

G. H. F. NUTTALL, M. D., Assistant, Hygienic Institute, Berlin.

A. P. OHLMACHER, M. D., Director of the Pathological Laboratory of the Ohio Hospital for Epileptics, Gallipolis, Ohio; Professor of Pathology in the Cleveland College of Physicians and Surgeons.

ISAAC OTT, M. D., Professor of Physiology, Medico-Chirurgical College, Philadelphia.

WILLIAM H. PARK, M. D., Assistant Director of the Research Laboratory of the Health Department of New York City.

FRANZ PFAFF, M. D., Ph. D., Instructor in Pharmacology, Harvard University.

W. T. PORTER, M. D., Assistant Professor of Physiology, Harvard University.

WALTER REED, M. D., Army Medical Museum, Washington.

E. T. REICHERT, M. D., Professor of Physiology, University of Pennsylvania.

WILLIAM T. SEDGWICK, Ph. D., Professor of Biology, Massachusetts Institute of Technology; Biologist, Massachusetts State Board of Health.

THEOBALD SMITH, M. D., Pathologist to the State Board of Health of Massachusetts; Professor of Comparative Pathology, Harvard University.

ALFRED STENGEL, M. D., Instructor in Clinical Medicine; Assistant Director of the William Pepper Laboratory of Clinical Medicine, University of Pennsylvania.

G. M. STERNBERG, M. D., Surgeon General, U. S. Army.

G. N. STEWART, M. D., Professor of Physiology, Western Reserve University.

W. S. THAYER, M. D., Associate Professor of Medicine, Johns Hopkins University.

E. L. TRUDEAU, M. D., Saranac Lake, N. Y.

W. GILMAN THOMPSON, M. D., Professor of Medicine, Cornell University Medical College.

IRA VAN GIESON, M. D., Instructor in Normal Histology, Columbia University; Director of the Pathological Institute of the New York State Hospitals.

J. W. WARREN, M. D., Associate Professor of Physiology, Bryn Mawr College.

F. F. WESBROOK, M. D., Professor of Pathology and Bacteriology, University of Minnesota.

JAMES H. WRIGHT, M. D., Director of the Laboratory of the Massachusetts General Hospital, and Instructor in Pathology, Harvard University.

CONTENTS

HOEN, A. G. On a Form of Degeneration of Striated Muscle met with in the Uvula 549

Plates XLII–L.

HEKTOEN, LUDVIG. The Fate of the Giant Cells which Form in the Absorption of Coagulated Blood Serum in the Anterior Chamber of the Rabbit's Eye 573

Plate LI.

WORCESTER, W. L. Regeneration of Nerve Fibres in the Central Nervous System 579

Plate LII.

WILLIAMS, HERBERT U. Experimental Fat Necrosis: The Action of Pancreatic Tissue upon Subcutaneous Adipose Tissue 585

Plate LIII.

LARTIGAU, AUGUST JEROME. A Contribution to the Study of the Pathogenesis of the Bacillus Pyocyaneus, with Special Reference to its Relation to an Epidemic of Dysentery . 595

MALLORY, F. B. A Histological Study of Typhoid Fever . 611

Plates LIV–LXI.

HIBBARD, CLEON M., and WHITE, FRANKLIN W. The Leucocytosis of Labor and the Puerperium 639

SMITH, THEOBALD. One of the Conditions under which Discontinuous Sterilization may be Ineffective 647

HIGGINS, CHARLES H. Notes upon an Epidemic of Fowl Cholera and upon the Comparative Production of Acid by Allied Bacteria 651

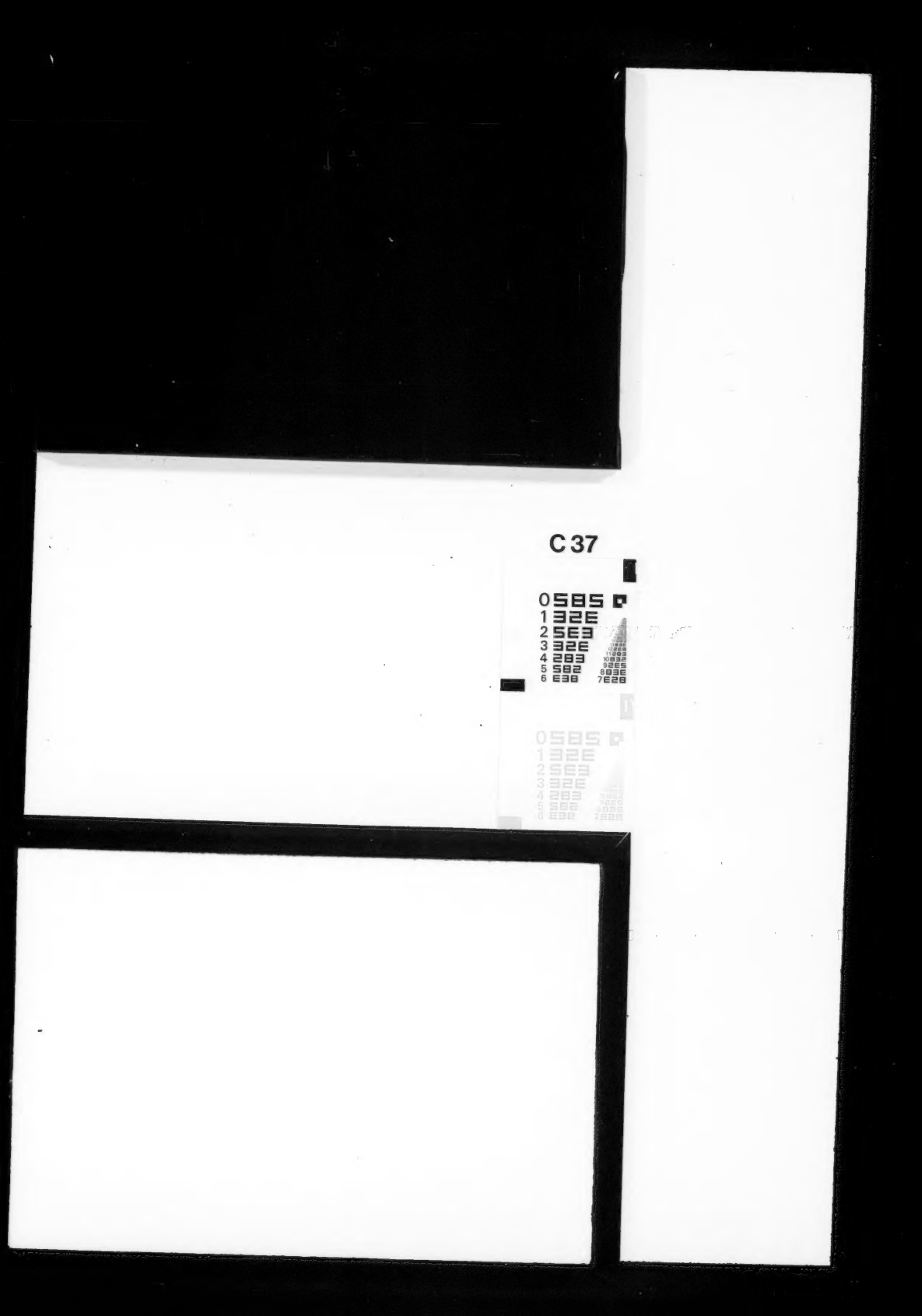
WEAVER, GEORGE H. Diffuse Sarcoma of the Cerebral and Spinal Pia Mater 669

The Journal of Experimental Medicine is issued bimonthly. A volume contains at least six hundred pages, with many plates and figures.

Papers for publication may be sent to the editor, Dr. William H. Welch, 935 St. Paul Street, Baltimore, or to any one of the associate editors. Other communications should be addressed to Mr. N. Murray, Johns Hopkins University, Baltimore, Md.

Contributors receive fifty copies of their papers free; additional copies may be obtained at cost.

The subscription price is $5.00 (£1,1,0; marks 21; francs 26) per volume. Subscriptions may be sent to the business manager of the *Journal*, Mr. N. Murray, Johns Hopkins University, Baltimore, or to the publishers, Messrs. D. Appleton & Co., New York and London. Mr. Gustav Fock represents the *Journal* at Leipsic. Messrs. Masson et Cie. represent the *Journal* at Paris.


C37